VOLUME 17 NUMBER 2

FEBRUARY, 1927

# Phytopathology

## An International Journal

## Official Organ of the

## American Phytopathological Society

CONTENTS

Significant [illegible] for Cuba; a progress report upon the root-disease [illegible] ..... J. A. [illegible] AND R. W. [illegible]

Zonate foot-rot of [illegible] cane ..... [illegible]

Nature of [illegible] of Marssonia spp. to Populus grandis ..... LEONARD W. [illegible] AND J. H. [illegible]

A new Alternaria disease of onions (Allium Cepa L.) ..... J. A. [illegible]

Additional hosts of Aphanomyces cuticles, the pea root-rot fungus ..... [illegible] B. [illegible]

Price { $5.00 per [illegible], United States and dependencies, Mexico and Cuba
{ $5.50 per year, Canada
{ $6.00 per year, elsewhere

PUBLISHED MONTHLY BY THE SOCIETY

OFFICE OF PUBLICATION

[illegible] AND GRANT STREETS, LANCASTER, PA.

Entered as second-class matter at the postoffice at Lancaster, Pa.

# Why pure chemical l

FOR greatest efficiency in spraying and dusting, the l
should be constantly high in c and low in
Ordinary lime are usually not uniform in analysis and
properties. Bell-Mine Pebble Lime is burned in rotary
is under constant chemical control. It analyzes 98.
oxide and less than 1% magnesia. Nationally recognized
logists endorse Bell-Mine Pebble Lime for spraying and

*Samples of Bell-Mine Pebble Lime for experimental purposes
sent free of charge. It is shipped in 180 lb. friction top
steel barrels which prevent slaking and assure its handling*

**AMERICAN LIME & STONE COMPANY**

# POTASH IMPORTING CORPOR
# OF AMERICA

PAYS

UNITED STATES DISTRIBUTORS OF POTASH SA
FOR THE
**GERMAN POTASH SYNDICATE**
GENERAL OFFICES
30 BRIDGE STREET, NEW YORK

BALTIMORE, MD.
CITIZENS' NATL. BANK BLDG.

ATLANTA, GA.
WOOD FERGUSON

Purchase from our advertisers. Mention Phytopathology when writing.

PHYTOPATHOLOGY

VOLUME 17 NUMBER 2

FEBRUARY, 1927

# SUGAR CANE ROOT DISEASE IN CUBA: A PROGRESS REPORT UPON THE ROOT DISEASE SITUATION IN 1925[1]

J. A. Faris and R. V. Allison

There has been much concern expressed by the cane growers of Cuba over what has been referred to for some time as "root disease." In the field it appears that this term has been widely applied to cane dying under a great variety of conditions, in fact under practically any condition that does not manifestly involve the above-ground parts of the plant structure. This tendency to bring every malady affecting the under-ground parts of the plant under the general term of "root disease" or "root rot" has been confusing and in many cases has resulted in planters throwing land out of cane over considerable areas because of a disease about which they know

Fig. 1. Successive stages in the death of cane affected with "root disease."

[1] Scientific Contributions No. 4, Tropical Plant Research Foundation. From the Cuba Sugar Club Experiment Station, Central Baraguá, Cuba.

nothing. However, it now seems that the disease in many of these areas is due to one or more of the soil conditions discussed in the second part of this report, and in several cases studies are being instituted by the planters to reclaim some of this land.

Investigations have been in progress in many of the more important cane-growing countries for many years, and everywhere the studies have been uniform and emphatic in pointing out the necessity for proper agricultural conditions for growing cane. In many cases what has appeared to be a serious disease menace has disappeared when some adjustment has been made in the matter of soil drainage, fertility, or cultivation. Owing to the confusion created by referring to such a large group of primary causes of disease as root disease, it seems desirable to briefly review the situation as we have found it at the end of our first year's work, with the hope that the

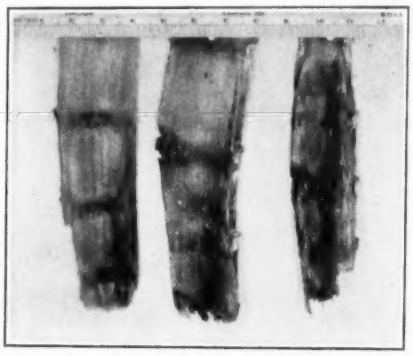


FIG. 2. Longitudinal section of the base of a cane stalk, showing characteristic foot rot due to *Melanconium*.

planters will study the failing areas in their fields in the light of this discussion.

In order to analyze the root disease situation and to facilitate the location of the initial weakening of the plants, the primary causes of the death of the cane are considered as due in part to pathogenic factors and in part to non-pathogenic factors.

The possible pathogenic invading organisms require further study for a determination of their nature, habits, and probable spread, but cannot be said, in the light of our present information, to be very serious factors upon any of the considerable number of plantations visited.

### POSSIBLE PATHOGENIC CAUSES OF ROOT DISEASE

The work upon this group of possible causes consists in the isolation of probable primary invading organisms and determination of their capacities for infecting healthy plants.

*Symptoms*

Plants dying of this complex malady exhibit certain rather marked symptoms. The outer leaves first show signs of drying and rolling up, while the inner leaves are still green. This is followed by rolling of the inner leaves and death of the outer ones. This drying may continue until all of the leaves are dry and the plant dies. In figure 1 is a series from a single hill which shows a gradual progress from a slightly affected stalk on the right (stalk 1) to one with leaves completely dry (stalk 4). The root systems of these stalks were poorly developed and the bases of the stalks themselves were found to be badly infected with the Melanconium species later discussed. Such plants are not uncommon in many of the fields of Crystallina cane in Cuba. These drying out changes may be brought about by any cause which cuts off the water supply from the top of the plant. In various parts of the Island, during both the wet and dry seasons, fields have been observed which contained plants in all stages of drying out. Usually the trouble is more conspicuous during the dry season, and any obstruction of the water flow would be reflected in the lack of moisture delivered to the growing parts at that time.

From the study of diseased cane plants in certain areas where decreasing production was attributed to root disease, it became evident that a very large number of these plants not only had diseased roots but also had developed what might well be called a foot rot (at the bases of the stalks). Plants affected with this basal stalk rot have a zone of dying tissue extending from the base of the plant upward. In some cases it is this progressing zone of diseased tissue which cuts off the water supply from the top of the plant, even though the root system is quite well enough developed to supply the needed moisture. There are several types of this basal stalk rot, at least one of which shows many indications that it is primarily due to an invading parasitic organism. This type of foot rot is described as zonate foot rot in another article.[2] The type under discussion here, pictured in figures 2 and 3, is associated with species of the fungus *Melanconium*, which proceeds from the base upward in the stalk, with an advanced zone of infected bundles, usually of a blackish color, extending through several internodes of the stalk. Figure 2 shows longitudinal sections of the base of an infected cane stalk with the blackened zone extending up one side. Figure 3 shows the brownish woody stem bases in which the water-conducting strands have been plugged up, thus preventing the functioning of the root system.

*Isolations*

As soon as the first two months' preliminary survey of the cane root disease situation in Cuba was finished, intensive work was begun upon the

[2] FARIS, J. A. Zonate foot rot of sugar cane. PHYTOPATH. **17**: 83–94. 1927.

fungi associated with these root and stalk rots. Isolations were made of a very large number of fungi from diseased tissues. After study and identification, many of these were eliminated as probable causes of the malady upon the grounds that they had been repeatedly experimented with by other investigators and found to be harmless to healthy cane. Among the fungi found in these tissues were two species of *Melanconium*; one of these, *M. iliau*, which causes the iliau disease of sugar cane in Hawaii, had not been previously reported from Cuba. It seemed quite probable that this might be an active parasite infecting the cane plants, although its destructiveness in the Hawaiian fields is attributed by Lyon to the tight binding of the leaf sheath of the young plants, thus preventing the development of the young shoots. The other, *M. sacchari*, as is well known, causes the ever-present rind disease. In view of its prevalence in these diseased plants, it seemed worth while to make some inoculations with pure cultures of this fungus and determine its pathogenicity. Since it was desired to follow the development of the root disease more closely, further experiments were carried out in order to insure infection in case neither of the Melanconiums proved to be capable of infecting the healthy plants. In these tests the seed piece was

FIG. 3. Bases of cane stalks infected with *Melanconium*.

covered with an abundance of finely chopped roots and stalk bases of diseased plants.

In connection with these experiments several different soils were used in order to determine, if possible, the influence of soil type upon the development of the disease. The soils were taken from spots where plants were dying from the disease. These soils were placed in sacks 2½ feet long and about 8 inches in diameter. One portion of the soil was sterilized. Lengthwise through the center of the sacks which were to be sterilized was placed a piece of rolled screen. This was done to facilitate sterilization but proved unnecessary, as perfect circulation of the steam was secured without it. Sterilization was done under six pounds steam pressure for three hours. One series of plants was grown in the soil without treatment, and the other series was planted in the same soil sterilized with steam.

FIG. 4. 1. Plants inoculated with *Melanconium iliau*. 2. Check, uninoculated. Soil I, sterilized. No infection.

*Method of Inoculation*

In the case of *M. iliau* and *M. sacchari*, the fungi were isolated in cane plug cultures, cornmeal agar, and cane juice agar, and these pure cultures multiplied in flasks. From these flasks blocks of one cc. were taken, placed upon a stick of Crystallina cane which had been sterilized in mercuric bichloride (1 to 1000) and rinsed in distilled water, and held in place by a piece of filter paper. Each seed piece had three nodes, but the two end buds

were removed when the center one was inoculated. These seed pieces were then planted in pots which were made by cutting gasoline cans in halves.

*Soil Types Used*

Four soil types were used in these experiments, and have been referred to by number in the tables. Type I (Havana clay) represents a heavy, ashy-gray to black, calcareous clay that passes down through calcareous clay of a lighter color into cream-colored chalk at an average depth of ten or twelve inches. In places, light-colored, slabby limestone occurs at shallow depth and not infrequently is strewn over the surface in considerable quantity, particularly where the topography is rolling. Where it is not too shallow, this type ranks very high as a cane soil, giving good yields and high sugar return under good cultivation. Type II, a deep red soil (Matanzas clay), derived from "dog-tooth" limestone such as is found in large areas in the provinces of Havana and Matanzas, is also an excellent cane soil. Type III (Oriente clay) is a very heavy black soil which, characteristically, is immediately underlain by cocó. This is ranked as a rather poor cane soil. Type IV (Limones clay) is a heavy, purplish-red clay, apparently formed

TABLE 1.—*The effect of inoculating Crystallina cane with Melanconium iliau. Cane planted March 6, 1925, and harvested January 1, 1926*

| Type of soil and treatment | Inoculations | No. of plants | Results |
|---|---|---|---|
| I. Sterilized | Inoculated | 56 | Free from root and stalk rots |
| do | Uninoculated | 6 | do |
| Unsterilized | Inoculated | 6 | do |
| do | Uninoculated | 6 | do |
| II. Sterilized | Inoculated | 9 | do |
| do | Uninoculated | 9 | do |
| Unsterilized | Inoculated | 9 | do |
| do | Uninoculated | 9 | do |
| III. Sterilized | Inoculated | 9 | do |
| do | Uninoculated | 4 | do |
| Unsterilized | Inoculated | 4 | do |
| do | Uninoculated | 4 | do |

from the breaking down of serpentine rock. In this the topsoil, upon drying, breaks into hard clods. This passes into pinkish red to red clay at 4–6 inches and into parent material (undecomposed or partially decomposed serpentine and other rock material) at 1–4 feet. It is only a fair cane soil but can be brought to fairly high production by good cultivation and the application of cachaza (filter-press cake).

*Results with Melanconium iliau*

In no case did *M. iliau* prove to be capable of infecting the growing cane plants, even when the organism was put over the buds at planting time. It should be pointed out that this is but one of the organisms found associated with root and stalk rot in the field. The plants were kept growing vigorously, as will be seen from the size of those in figure 4. A portion of the plants which had been inoculated with *M. iliau* was cut to the ground surface and these plants will be carried to the first ratoon. The pots are very small and the soil is filled with a dense mat of roots, so the plants are developing against great odds.

*Results with Melanconium sacchari*

A second series of experiments was carried out in a similar way with the rind disease fungus, *M. sacchari*, which was always associated with the foot rot of a seedling cane. The fungus was isolated from blocks of diseased tissue, increased in pure cultures in cane juice agar, and blocks of the actively growing fungus were placed over the young cane buds at the time of planting. Only one soil type was used in the experiment, results of which are summarized in table 2.

Here again the results indicate that this fungus is not able to infect healthy, vigorously growing Crystallina cane. Figure 5 shows some of the plants in this series.

FIG. 5. 1. Plants inoculated with *Melanconium sacchari*. 2. Check in sterilized soil.

TABLE 2.—*The effect of inoculating Crystallina cane with Melanconium sacchari. Cane planted March 23, 1925, and harvested January 7, 1926*

| Type of soil and treatment | Inoculations | No. of plants | Results |
|---|---|---|---|
| I. Sterilized | Inoculated | 24 | No sign of root or stalk rot in any of the plants |
| I. Sterilized | Uninoculated | 4 | do |
| I. Unsterilized | Inoculated | 6 | do |
| I. Unsterilized | Uninoculated | 6 | do |

*Inoculation Experiments with Various Organisms*

In this series of experiments the bases of ratoon Crystallina plants dying from root disease were chopped into small pieces and the material used to inoculate the seed pieces. The buds were covered with a quantity of the chopped material, which was held in place by moist filter paper. The soils were taken from diseased areas where considerable complaint of losses from root disease had been made. Soils I and IV were used in this series and were treated as in the previous experiments. The results are summarized in table 3.

Here, again, when diseased tissue was used for inoculations so that any parasitic fungus present might have opportunity to act, no signs of the root disease developed (Fig. 6). The plants were removed from the small cans,

TABLE 3.—*The effect of inoculating Crystallina cane with chopped diseased cane roots and stalk bases. Cane planted March 23, 1925, and harvested January 8, 1926*

| Type of soil and treatment | Inoculations | No. of plants | Results |
|---|---|---|---|
| I. Sterilized | Inoculated | 18 | No sign of infection |
| I. do | Uninoculated | 4 | do |
| IV. do | Inoculated | 8 | do |
| IV. do | Uninoculated | 3 | do |
| IV. Unsterilized | Inoculated | 6 | do |

the soil washed away from the bases of the stalks, and a careful inspection made of both the roots and the stalks. The latter were split down to the seed piece. The soil was washed from the roots of typical plants from some of these experiments. The root systems of these plants are shown in figures 7 and 8.

*Discussion of Results*

Since the type of root disease under experimentation in these three series of cultures is that characteristic of a very large percentage of the troubles of

this nature all over the Island, and since the preliminary experiments failed to show any primary invading organism, we must analyze the problem somewhat further to see if we can arrive at the key to the situation. It is evident that we are dealing with a variety of cane-growing conditions, but in the end the invading organisms, with some exceptions, are the same. These organisms we find to be semi-parasitic in that they invaded only weakened tissues and thus were not capable of infecting the healthy plants in the experiments. Therefore it becomes evident that in all of these soils we have some factors operating to injure or weaken the plants. Field studies in various parts of the Island have indicated some of these causes contributing to the dying out of the cane, the more important of which are discussed later. The term root-rot, which has been so commonly applied to this dying out of cane, covers a very large number of primary causes for the weakening of the plants, which are then invaded by fungi which could not otherwise attack them. In realization of this fact, which has been established by experimentation and field observation, the first thing to be investigated when plants in the field begin dying out is whether the trouble may not be accounted for by one of the reasons discussed below. We do have some cases which cannot be thus accounted for. These are under investigation, but the experiments have not progressed far enough to be reported upon except to describe the occurrence of the disease, its effect upon the plants, etc.

FIG. 6. 1. Plants inoculated with material obtained from chopped diseased cane roots and stalks. 2. Check, uninoculated.

NON-PATHOGENIC CAUSES OF ROOT DISEASE

Under non-pathogenic factors will be grouped a number of conditions, essentially ecological, which are of direct concern in determining the physical and chemical fitness of the soil environment to the health and development of the roots of the cane plants. While any of the factors mentioned below may be individually active and are discussed more or less individually, it is seen that in a given case in nature a number of them may be simultaneously involved in the development of a given condition in the soil which, for several reasons, may be extremely deleterious to plant growth. Furthermore, as will also be discussed, some of these factors may be active in a more or less alternate fashion and in this way insure a more continuous injury to the health of the plants. This might well be instanced in cases where the soil is especially subject to conditions of flood and drought. Thus an intricate complex series of factors frequently must be taken into account, as well as the individual conditions referred to, in considering the health and development of the cane plant in any particular case. The enumeration and discussion of the more outstanding of these conditions which so frequently constitute the primary cause of the so-called root disease in cane represents the purpose of this part of the report.

*Lack of Drainage*

In point of area concerned, lack of or deficient drainage is one of the most important reasons why cane can not be grown to any extent in the Island.

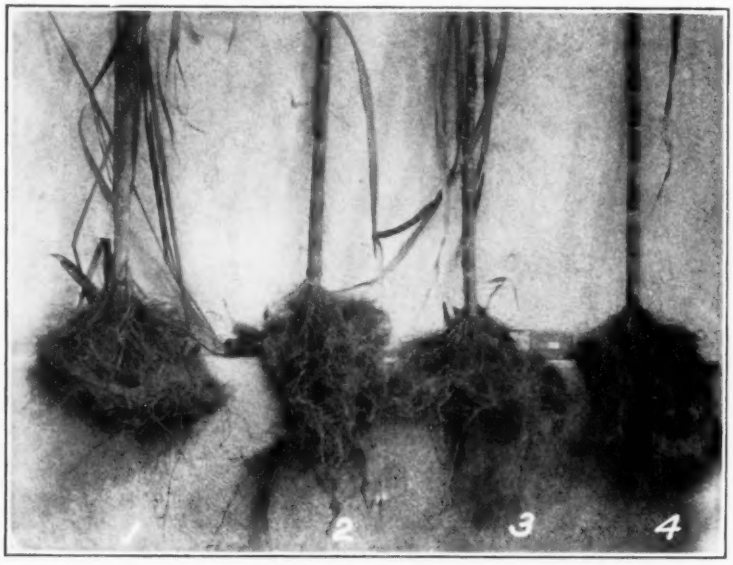



FIG. 7. Root systems of plants artificially inoculated. 1. Soil III, inoculated with *Melanconium ilau*. 2. Soil II, inoculated with *Melanconium sacchari*. 3. Inoculated with chopped diseased cane roots and stalks. 4. Uninoculated check.

This condition is particularly prominent in the regions of the heavy brown and black impervious soils of the coastal plains and river flats throughout the country, especially in the Province of Oriente.

In our observations, extensive areas are encountered very commonly where the question of economic production upon the soil involved has absolutely no basis for discussion or consideration until an efficient system of drainage is supplied.

In picturing a plant struggling along in such an environment it must be remembered that every living cell in its tissues is entirely dependent upon the life-sustaining process of respiration. This is dependent, in turn, upon ready accessibility to air. Imagine the anaemic nature of the process in one of these suffering plants with its roots buried in sticky, soggy clay, where at times the whole of the soil surface is flooded with free water through periods perhaps of several days or weeks. Plants dying under these conditions of strangulation have been frequently referred to as victims of root rot.

It has been found that the drainage problem is further complicated over rather extensive areas, particularly in Oriente and upon the Coastal Plains in the other provinces, through the presence of common salt in toxic concentration. In certain areas adjacent to or recently influenced by the sea in this and other provinces, the impregnation of these heavy clays with salt is so great that it frequently appears as surface incrustations upon the banks of

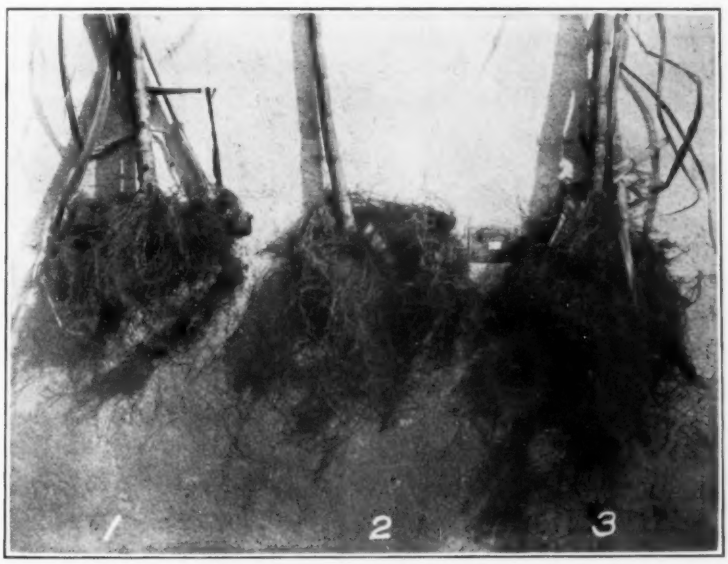



FIG. 8. Root systems of plants artificially inoculated. 1 and 2. Soil IV inoculated and uninoculated with chopped diseased cane roots and stalks. 3. Soil II inoculated with *Melanconium iliau*.

shallow ditches or on the bottoms of evaporating pools. Samples of the soils have been analyzed from these general regions which show a total salinity as high as 0.3, 0.4, and even 0.5 per cent. The concentration is usually found to increase with depth. In terms of total salts the incrustations referred to as being left by small evaporating pools contain as much as 5 per cent as sampled. Salt flats in positions adjacent to the sea and bearing characteristic vegetation in the form of shrubs, grasses, and weeds quite commonly have a salt concentration in the top soil of 2 per cent. While this is manifestly not a cane soil, we have seen cane planted in such positions in actual competition with mangrove and salt willow.

As indicating the rather general condition in this connection, the concentrations given in table 4 were found at the depths indicated in a fairly heavy coastal clay in the south of Havana Province. Here the surface soil was a dark brown clay, passing at two inches into a lighter brown plastic clay, and at 5 inches into yellow plastic clay. This became heavier and more impervious with depth, and a faint gray mottling appeared at about 44 inches. These samples were taken early in 1925 and the analyses made by the Bureau of Soils in Washington.

The present discussion is concerned primarily with such concentrations of salts as will visibly affect the health of the plant through toxicity to grow-

TABLE 4.—*The concentration of salt at certain depths in a heavy coastal clay soil in southern Havana Province, Cuba*

| Sample no. | Soil depth in inches | Salt in per cent |
|---|---|---|
| 32659 | 0– 2 | 0.21 |
| 32661 | 5– 8 | 0.41 |
| 32662 | 8–44 | 0.67 |
| 32664 | 56–65 | 1.04 |

ing roots. Injury from such a source of course predisposes these organs to natural rotting. Active toxicity of this type has been observed in several areas. In the case of the cane plant, however, it is highly important to understand that the tonnage of the crop removed from saline soils gives no assurance of a normal rendement of sugar in the mill. For, while the salt may be present in sub-toxic concentration in the soil and the plants develop in an apparently normal manner, its accumulation in their juices may still be such as to hinder the crystallization of sugar in the mill, and a high production of molasses will result.

Similarly, waters from the ditches or wells of some of these regions are heavily charged with salt and frequently brackish. Waters from open ditches have shown as high as 11,000 parts per million in terms of total salts. While on these areas the absolute drainage conditions may vary somewhat, either locally or in general, from those discussed above where the

salt factor is absent, the solution for their development is absolutely the same, namely, drainage. For unless ditches are opened for the natural washing out of these readily soluble salts, the situation may be expected to become increasingly serious with continued cultivation. Under such conditions, the plants, with the natural handicap of the presence in solution of a toxic substance, usually make less growth or perish more quickly than where deficient drainage alone is a factor.

Besides the direct effects of these drainage conditions upon the plant, their damage is reflected indirectly through the effect of the changed physical structure and aerability of the soil mass upon the development and activity of the soil microflora. This, as noted, is produced through the lack of aeration brought about by the general degeneration of the physical condition of the soil structure as well as the presence in certain areas of the toxic salts indicated. The depression, or loss in this manner, of the activity of the various important groups of the soil flora in their effective preparation of certain of the soil constituents for assimilation by plants must also be regarded as a serious matter.

*Lack of Moisture*

In contrast to the conditions discussed under lack of drainage, moisture deficiencies are usually most commonly associated with the pervious red soils so far as the soil types of importance in cane production are concerned. To be sure, serious difficulties of this nature are also experienced in the lighter sabana soils when they are cultivated. This is due largely to the fact of their extreme permeability and comparatively small capacity for retention of available moisture. The moisture situation in this respect is complicated by the alternation, in seasons of approximately six months each, of rainfall and drought. Thus the cane upon soils that are weak in their ability to store water may naturally be expected to suffer greatly during this extensive period of rainfall deficiency. Furthermore, certain types of red soil appear to dry out much more than others. Thus in February of last year, 1925, in certain areas of a red soil in Camagüey, no apparent moisture was encountered even at a depth of 40 inches. The soil was so dry that it could be brought to the surface, even from this depth, only with great difficulty, since it powdered under the soil auger and fell back into the hole as the instrument was withdrawn. This has since been found to be the usual condition of this soil during the dry season.

Upon a soil of this type the moisture deficiency referred to is regarded as of first importance, and the protection of the plant in this respect, particularly by methods of planting and subsequent cultivation and fertilization, is a most important consideration.

Moisture deficiencies also appear during the dry season upon the heavy brown and black soils, referred to previously as usually suffering from lack

of drainage. Here the results are frequently very serious; in contrast to the general deportment of the red soils, the brown and black soils usually crack very badly. This, in addition to producing serious physical damage to the roots of the plants, exposes a much greater surface of the soil, and particularly the deeper layers, to evaporation. The desirability of preventing this tendency both for the protection of the roots of the plants from physical injury and desiccation and the conservation of moisture in the soil is readily apparent and will be discussed further.

In either case the weakness imposed upon the roots of the plants by such a long period of desiccation is a very serious handicap to their immediate and future development. The mutilation of these organs in the fashion indicated also leads to an undue exposure of these parts to the natural microflora of the soil, which is ever seeking ready sources of energy of the type represented in abundance by the carbohydrates in these structures. Consequently, if the conditions are too rigorous, the roots are certain to decay.

There are extensive areas in the western part of the Island where this condition is to be very commonly observed, either in patches or in whole fields. Here the stand of cane exhibits varying degrees of degeneration. It is a noteworthy fact, and one that has been demonstrated in several instances, that where these areas have been cultivated and planted according to practices in keeping with rational agriculture, the trouble disappears entirely and good yields result. It is a fact truly worth keeping in mind that the manipulation of the available supply of moisture is a most important consideration in the cultivation of cane in Cuba. However, where plants have been overcome by this general condition, and their entire root system practically destroyed by the drying out and cracking of the soil, the trouble is frequently referred to as root rot.

*Deficient Cultivation*

The term cultivation is here used to include practices in the preparation of the seed bed, the planting of the seed, and the treatment of the soil subsequent to the appearance of the young plants, whether plant cane or ratoon, previous to the closing of the middles.

It is obvious that cultivation in this sense is very intimately related to the conditions of drainage and moisture discussed above. In the case of heavy impervious soils the chief significance lies in the matter of plowing in banks and level planting, while in the instance of the pervious red soils it is deep plowing and deep planting. Observations have been made upon plantings under good conditions of cultivation in both cases, however, which indicate the desirability of some very fundamental studies in root range, particularly in relation to varietal adaptability before too specific recommendations can be made in this connection.

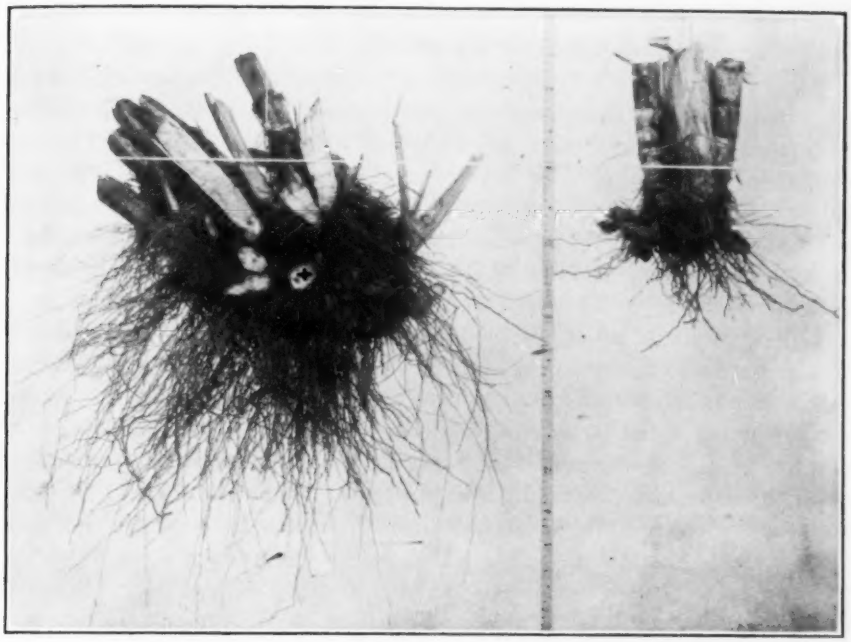


FIG. 9. Roots of cane, showing the effect of planting and cultivation on root development. The white line indicates the original position of the soil level. The figures on the tape indicate decimeters.

Whether upon porous red soil or the other extremity, the impervious brown and black clays, proper cultivation or other practices productive of the same results subsequent to the appearance of the young plants is an absolute necessity in the control of grass and weeds. Herein is one of the great dangers of deficient drainage, for the excess of moisture which promotes the growth of obnoxious weeds in the cane fields simultaneously prevents the application of such cultural practices as will not only destroy this growth but assist very materially in the maintenance of a proper physical condition of the soil.

In the majority of cases this can best be done by moving the trash to alternate middles, or, where the trash is light, from two middles into a third, and cultivating those exposed. By such a procedure a sufficient trash covering is put upon one row to keep down the weeds, while in those exposed they are destroyed by cultivation. In the case of the heavy, stiff, black clays, this procedure serves not only to destroy the weeds but to reduce the drying out and cracking of the surface and sub-surface soils and consequently effectively to prevent the physical injury to the roots which these conditions produce. It was largely through the development of such a pro-

gram of cultivation that the successful restoration of the declining production on the red soils of western Cuba was first locally demonstrated.

Figure 9 shows the difference to be expected between poorly planted and cultivated canes and those receiving the treatment described. The stool on the left represents primavera (spring) cane at one year of age. This was planted in April, 1924. The cane piece shown in cross section in the central part of the root mass represents the original seed piece. Two months before the picture was taken, when the cane was less than 10 months old, a test cutting showed a yield of 97,000 arrobas of net cane per caballeria (37 short tons per acre). The roots on the right represent one of the best stools in the first ratoon of an improperly planted and improperly cared for field. Here at the first cut from primavera the yield was slightly less than 25,000 arrobas per caballeria (9.5 tons per acre). At the time of taking the picture the stand in the latter case was showing all the conventional signs of root rot, being very irregular and having many patches and areas from which the cane had practically disappeared. Taken as a whole the crop was scarcely worth cutting. At the time of harvest the weight of net cane in the former stool was 84½ lbs., while that of the inferior stool was 5 lbs.

In matters of cultivation, the manner of cutting the cane at the time of harvest and the method of applying fertilizers are of considerable importance in relation to the control of the depth of root development. This is made apparent by the illustrations in figure 10. The stools were taken from a burned-over area on an average red soil in Camagüey Province, and show the extreme superficiality of the root systems that may develop after a succession of annual cuttings. These stools were removed after the eighth cutting from cane that had not been cultivated since planting in virgin soil after burning the original forest growth. The tendency is especially well shown in B, where the buds that germinated were exceedingly superficial and the general condition and position of the root system in relation to the surface of the soil (indicated by the white line) is very bad.

Attention is called to the shallow dead stems on the left in figure 10, A, with the rootstock passing from beneath these to the right. It is seen that the maximum depth of this part is slightly more than 4 inches. There were a total of four plants in this stool. Two other stems sprang from the upper part of the rootstock, but were cut away to show the connections more clearly. The shallowness of the prospective root system is apparent. The general condition in B is particularly bad in this respect. Note that the stalks on the left (two smaller ones cut away from front) spring from top of old stem to right, also the larger stems to the right. This old stem is seen to be rotting down the center to within an inch of the dead rhizome of the year past. Parts of the lower section of the young plants were also becoming discolored at the time of examination. It is a point of importance in connection with the plants here discussed that in the general area of red

soil from which they were taken, infestation by root mealybug is particularly common and frequently bad. In the immediate area from which these stools were taken an examination of the roots of more than 50 stools showed an infestation of 100 per cent.

The tendency for the stool to rise, as referred to above, may also be accentuated by fertilizing methods. In the application of the fertilizer materials it is recognized as the better procedure in the case of ratoon cane to make a desaporque (furrow turned from along rows toward middle) along both rows in the exposed middle and spread the fertilizer along the exposed bases of the cane stools. After this application the soil should be worked back into the furrow by subsequent cultivation. The importance of the offbarring in cutting away the old root structures and aerating the soil mass about the living centers of the cane stool is readily apparent and has long since been established in Cuban agriculture.

The application of the fertilizer in the bottom of such furrows rather than on the top of the stool is to be no less emphasized. By so doing the material is placed in a position to be immediately available to the newly

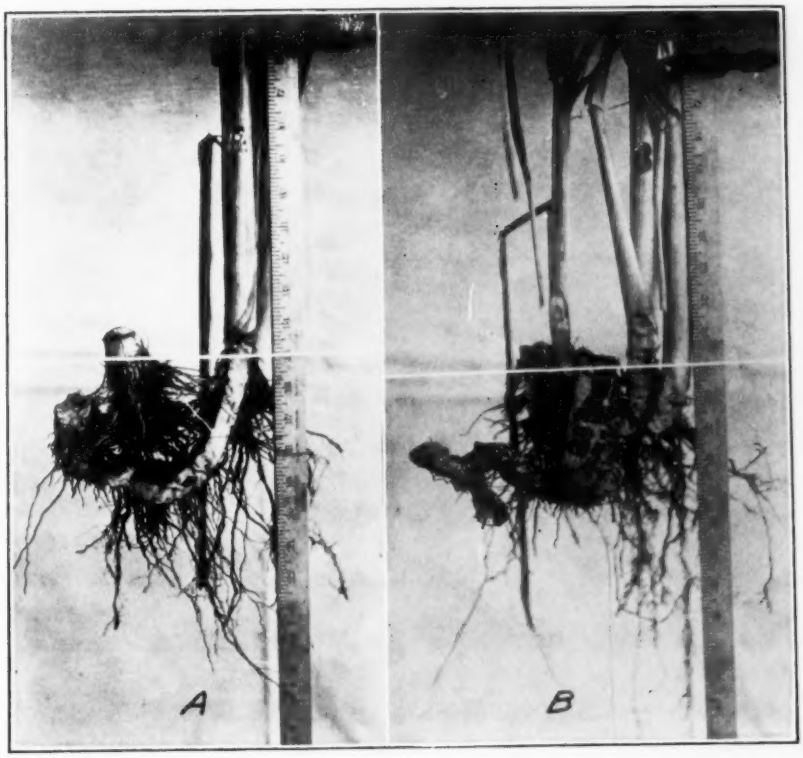



FIG. 10. Photographs A and B showing the superficial nature of roots of ratoon plants taken, after eighth cutting, from burned over area on red soil, Aug. 19, 1925.

FIG. 11. Cane growing upon good red soil in Matanzas Province on opposite sides of same guardarraya, although on different colonias. A. No fertilization, no cultivation; B. fertilized and cultivated.

formed roots of the plant. By virtue of its position, the tendency also will be for their development downward rather than upward, as might be expected where it is placed superficially above their normal zone of growth.

The general degeneration in the stand and yield of cane as the result of deficient cultivation is well shown in figure 11, A and B. These two fields are located upon opposite sides of the same guardarraya, and the soil, identical in both cases, is of the good deep red Matanzas clay type derived from limestone. These fields, though adjacent, are upon different colonias, and the tremendous difference in the cane stand is due to fertilization and cultivation. The grass-ridden cane shown in A is not an instance of root disease or root rot, as frequently has been inferred under similar circumstances. It is simply an excellent though unprofitable demonstration of very poor farming, where this condition develops as a consequence of the fact that the preparation of the soil, the planting of the seed, the application of fertilizer, and consequent cultivation of the cane has been in entire disregard of the factors here discussed.

*Deficient Fertility*

It is generally understood that deficiencies in soil fertility in Cuba are more likely to be encountered in the older agricultural areas of the West. It is not to be doubted, however, that careful studies in relation to soil type will indicate specific deficiencies of a more or less local nature to be generally distributed throughout the Island. Since outstanding deficiencies in any of the more important elements necessary for plant growth are known to be the reason for a greatly reduced vigor and resistance, disturbances of the nature generally discussed above must be expected to appear when the soil solution to which the plant has access becomes unbalanced in this respect and one or more of the elements becomes unavailable.

The reduction of the vigor of the plants, and consequently the severity of attack of the fungi and bacteria of the soil upon the weakened roots, will thus be more or less directly determined by the extent of the deficiency involved. Deficiencies of this nature, particularly in relation to phosphoric acid and potash, have been studied in connection with both corn and cane for several years. In fact, it is now definitely thought that the so-called resistance to these conditions among certain varieties is nothing more nor less than a lower requirement for the element of which other susceptible varieties find an inadequate supply in the same environment. This may be due either to natural differences in feeding power for the same materials or to actual differences in requirements. The latter appears the more plausible as between varieties.

While the outstanding elements which the plant must draw from the soil for the elaboration of its food are nitrogen, phosphorus, and potassium, it may happen that still others are present in deficient quantities. They may

also be present in such unbalanced proportions as to develop either a toxic condition or one that is suboptimum to the assimilative interests of the plant in one way or another. Thus it is possible that in some soil types there may be a positive calcium deficiency. In others the presence of an excess of magnesium over calcium may repress the activity or availability of an otherwise abundant supply of this element even though it be present. This latter condition seems to be the case most frequently in soils derived from serpentine and is well displayed upon bibijagua (leaf cutting ants) mounds, where the toxicity, apparently due to the material brought up from the subsoil, persists even after the mound has almost disappeared as a result of cultivation. In certain of these soils the Ca/Mg ratio has been found to be 1 : 3 or even wider; in fact, comparatively high concentrations of magnesium have been found along with but a trace of calcium. A further consideration will be given this matter in connection with the various types derived from this material as well as with other types when more analyses are available. On the other hand, where the soil is deficient in calcium and the material brought up by these insects is calcareous, the benefits to plant growth are at once apparent in the better growth and color of the cane upon these spots. This contrast of effects is commonly to be observed in one way or another upon soils of this type. In the case of those having the injurious type of subsoil referred to, the natural outcrop of this material is frequently the cause of local to fairly extensive sabana areas.

Manifestly the only logical solution of this general phase of the problem is a systematic study of varietal requirements in relation to soil type. At the present time, however, upon areas where the cane is doubtless suffering from specific deficiencies of this nature, in complex with one or more of the conditions discussed above, the impaired stand and dying cane is sometimes attributed to root rot.

*Other Factors*

In a substratum possessing such native complexity as the soil, there is a great variety of factors that might appear to disturb the metabolism of living plants which are directly dependent upon it for a vital part of the raw materials from which their food and consequently their whole structure is elaborated.

The availability of the more common elements has been emphasized. The importance of their balance should also be noted. In this, lime should be included, for it is important not only in the maintenance of a desirable reaction and physical condition of the soil but calcium is among the more important elements necessary in the metabolism of plants.

Soil acidity is also an important consideration in many cane producing countries, but in Cuba the soils used for cane are, for the most part, slightly or actively alkaline. Elsewhere under conditions of excessive acidity, it has

been recognized for several years that the release of such toxic substances as aluminum, manganese, or iron has a deleterious effect upon plant growth; and, in relation to cane production in Hawaii and corn production in certain parts of the United States, this problem is receiving considerable study.

In the matter of definite soil type peculiarities that require attention in connection with general plant response, those associated with what has been tentatively classed as Oriente clay should be considered. In this the topsoil is a heavy black clay high in organic matter and usually low in lime. It cracks badly on drying and is underlain at 5 to 16 inches with a white chalky lime sometimes mixed with argillaceous material or in combination with hard or semi-hard limestone. It is a matter of common observation in many parts of the Island that this chalky material when brought into the zone of root development is very deleterious to plant growth. In fact, when the growing roots come in contact with this stratum they are said to turn quite upward and away from it. The appreciation of this effect is so general that in some instances deep plowing is assigned as the cause of crop failure in areas where this chalk could not possibly be reached with the plow. In such cases the degeneration of the cane was doubtless due rather to an impaired physical condition of the soil as a consequence of its improper handling. As noted above, cane is also commonly observed to do badly on many of the soils derived from serpentine. In some it will scarcely grow at all. Information at hand at the present time on the specific nature of the active principle involved in such instances is only sufficient for conjecture. Systematic studies in the laboratory and in the field upon this, as upon other broader relations, should yield information of the greatest importance, for analogous peculiarities in the interrelation of plant to soil type are to be found in other instances.

In the matter of simple physical injury to the living roots of the cane as a predisposing circumstance or condition to the entrance of the normal saprophytic fungi and bacteria of the soil into these structures, the situation is seriously complicated by certain soil-inhabiting insects that infest and feed upon these underground parts of the plants. Those that have been most commonly observed in this connection under field conditions in Cuba are the root mealybug, the white grub, termites, certain root borers, and, in very limited areas, wireworms. Any of these, through their natural feeding habits, open the plant to secondary parasites that commonly do much more serious damage than the original wounds inflicted by the insects themselves. In studying a definite situation involving failing or dying cane which has the appearance of root rot, therefore, care should be taken early in the examination to ascertain whether attacks of this nature may not represent the primary source of injury, due consideration of course being given to the possible cyclic or seasonal nature of their appearance in each instance.

Furthermore, important correlations are found to exist between soil type and the facility with which the infestation by some of these insects proceeds.

In general the relation is one of pulverulence or natural mulching (polvillo) upon drying. This point will be touched upon in some detail in a later paper. In this connection it is of interest to note the important part which snails are reported to be playing in this same rôle upon the roots of the cane in Louisiana.[3] No significant infestations of this type have been observed by the writers in Cuba.

In regard to the irregular condition of the cane that is frequently found along the margins of the fields adjacent to the guardarrayas (firelanes) and particularly at the corners, it has been observed that this condition of the external growth is considered altogether too frequently as an indication that the entire field is suffering from root rot. It is found, however, that the more common cause for this condition is the promiscuous turning into the fields of the heavy cane carts, particularly during rainy weather; the foraging of animals, either while at work or at large; and also the simultaneous or subsequent encroachment of grass and weeds from the guardarraya. Where such parts of the field have been passed over repeatedly by carts and the soil thoroughly packed and in poor physical condition generally, considerable difficulty is frequently experienced in re-establishing the stand of cane. As noted above, this irregular condition of cane brought about either through physical injury from carts or bulls or through competition of weeds, or both, has been frequently referred to as root rot.

## SUMMARY

The terms "root disease" or "root rot" have been applied to the dying of sugar cane under a variety of conditions, which are grouped as pathogenic, due to fungi and other parasitic organisms, and as non-pathogenic, due to lack of drainage, lack of moisture, deficiencies in fertility, cultivation, and other factors.

Of the various fungi isolated from diseased cane stools, two species of *Melanconium* were suspected of having a causal relation to the injury, but inoculations of cane plants in pots of sterilized soil with pure cultures and with decaying rootstocks gave negative results.

The field studies showed root disease to be associated with lack of aeration in undrained soils, with high salt content of the soil, with drought and resultant cracking of the soil, with high cutting and surface application of fertilizers, with infertile soils, and with the attacks on the roots of several insects and other small animals.

Improved agricultural practices are indicated as the most important means of relief.

TROPICAL PLANT RESEARCH FOUNDATION,
CUBA SUGAR CLUB EXPERIMENT STATION,
CENTRAL BARAGUÁ, CUBA.

[3] RANDS, R. D. Root disease of sugar cane in Louisiana. U. S. Dept. Agr. Circ. 366. 1926.

# ZONATE FOOT ROT OF SUGAR CANE[1]

JAMES A. FARIS

During the process of investigations to determine the primary causes for dying out of sugar cane plants (*Saccharum officinarum*) in the sugar estates in Cuba, a foot or basal stalk rot has been found which does not seem to have been previously recorded or described. This disease was first observed by the writer in parts of Oriente province in November, 1924, in spring planted cane of about eight months growth. During 1925 the disease was again found in the western part of Camagüey province upon stubble cane of the third ratoon. In each case the disease was quite

FIG. 1. Stool of sugar cane affected with zonate foot rot. Note the size of the stool and the number of shoots, many of which are dead.

[1] Scientific Contributions No. 5, Tropical Plant Research Foundation, from the Cuba Sugar Club Experiment Station, Central Baraguá, Cuba.

widely distributed over considerable areas within the immediate localities.

In the pathological collections of the Cuban Experiment Station at Santiago de las Vegas, a portion of a stalk of cane infected with this disease is reported[2] as having been sent to the station by a mill manager in Oriente province September 28, 1923. The present known distribution of the disease is therefore confined to widely separated localities in the provinces of Camagüey and Oriente, Cuba.

### DESCRIPTION AND GENERAL CHARACTERS OF THE DISEASE

In both cases where this disease has been observed in the field, the dying plants were surrounded by very strong, vigorously growing cane. In the first instance a few hills along the edges of the fields were observed to be wilting, and a close examination of these stools revealed a large number of dead shoots (Fig. 1). After the trash had been cleared away it was found that every stalk in the stool was diseased, and that some of

FIG. 2. Portion of a hill of cane affected with zonate foot rot.

[2] Personal letter from Stephen C. Bruner, Pathologist, Estacion Experimental Agronomica, to the author.

the larger stalks seemed to be dying rather suddenly. Such infected plants may occur singly or in spots of several hills. In one case an area was observed where six hills were dead. In another case two healthy plants were found entirely surrounded by plants dying of this zonate rot. In the primavera or spring cane the diseased plants were inconspicuous at first, as they were about the same height as the healthy cane. Whenever the foot rot had progressed far enough in a considerable number of stalks to stop the water supply, the hills presented the noticeably uneven, ragged appearance shown in figure 1.

Figure 2 is a photograph of a portion of a stool of cane which was killed by the zonate foot rot disease. This hill of cane produced 19 cane stalks which reached a good size before succumbing to the disease. Note the size of the stalks, many of which were 1½ to 2 inches in diameter. No cane suitable for milling was harvested from this stool. The pithy hollow character noticeable at the tops of the stalks is often found in plants affected with this disease. This I attribute to the continued drain upon the moisture of the stalk after the root system has ceased functioning. Thus the stalk is dried out and becomes pithy and hollow between the nodes preceding decay from secondary fungi or bacteria. In some cases there is a central cylinder of brownish tissue whose color seems to be developed through chemical changes and degeneration of the cell walls of the center of the stalk. Surface sterilized plugs have been taken from this brown tissue above the area showing the zonated dry rot and plated in cane juice agar, but no organisms have developed.

FIG. 3. Surface view of a portion of a cane stalk affected with zonate foot rot. This shows the clear surface markings.

FIG. 4. Longitudinal section of the base of a cane stalk showing the characteristic zonate foot rot. At A is a root in which the cortex has been decayed.

## SOIL TYPES CONCERNED

The disease has been found in considerable quantities upon two very different types of soil, one the Saltanejo or "hog wallow" soil of Oriente province, and the other a dark chocolate red soil of the Matanzas clay type. The former of these is very heavy black soil with a clay subsoil, and the latter is a porous red soil. Since these types are very different in their physical characteristics, soil solution, etc., there is little indication that the disease is limited to any great extent by soil type.

## APPEARANCE OF DISEASED STALKS

At the bases of invaded stalks we find a zonate dry rot which leaves distinct rings of a blackish brown color upon the rind at that point. These ring-like zones are usually concave in form and more conspicuous above each node in the base of the diseased stalk. They are often obscure

until the wax coat of the stalk is removed, when they are very striking in appearance. Such zones are characteristic of no other known cane disease and serve as an easy method of separating this from other similar cane troubles.

Upon cutting either a cross or a longitudinal section of the stem it will be seen that these conspicuous zones on the rind are but the continuation of characteristic zones in the pith of the stem. Figure 3 shows these zones on the outer part of the rind. They tend to be concentric, but wider apart at the top the farther they are from the node.

A longitudinal section of the base of the stalk shows well defined alternating regions of gray and reddish tissues. The reddish bands are usually narrower than the gray, but both zones vary considerably in width. These zonated areas are below ground or within the first few inches above the soil surface. The cane cutters cut such stalks above the dry, woody portion; hence canes from the diseased areas might not indicate the presence of the infection. Figure 4 is a photograph of a longitudinal section through the base of a cane stalk with the characteristic zoning of the tissues. The alternating gray and reddish layers are more striking in appearance in fresh material than in the photographs. They are also more conspicuous in infected stalks which are still alive than in older dried stalks, as in the latter case the reddish zones may have almost faded out. In such cases the color usually remains in the more fibrous parts of the stem. The marks most persistent on the dead canes are the zones formed on the rind of the stalk.

The ring bordering upon the healthy tissues of the stem is a deep blood red in color. This is separated from the ring below by a somewhat wider grayish zone, and this in turn is followed by a brownish red zone. These zones fade to a brown or buff color when the tissues are completely dead. Above the last root ring shown in figure 4 the stalk was sound and showed no signs of rotting. However, the plant was wilting from the lack of moisture. It had a sufficiently well developed root system and plenty of rain had fallen to supply moisture, but the roots could not function because of the obstruction to the upward passage of the water by this dry rot.

Figure 5 shows the base of a stalk with an excellently developed root system, the lower part of which had lost its power to function because the woody zonated tissue prevented free communication with the stalk. This plant has tried to overcome the decreased supply of water by pushing out roots from the higher nodes. The disease has progressed to the node indicated by the arrow. Above this point the tissues of the stalk have every appearance of normal tissue. This sharp differentiation between the diseased and the healthy tissues by the advancing zone is characteristic of the rot.

FIG. 5. Longitudinal section of a sugar cane stalk affected with zonate foot rot. The arrow indicates the most advanced zone of infection.

A cross section of infected stalks shows the zonated condition to a very marked degree. Here the zones roughly approach concentric circles in form. The zone bordering the healthy tissue is a deep red color, and there is a marked tendency of the color to fade as the tissue rots. The infected tissue is dry and woody and thus in marked contrast to the fresh sappy pith of the portion of the stem not yet affected. Figure 6 is a photograph of sections of four infected stalks.

### ENTRANCE INTO AND SPREAD THROUGH THE STALKS

The chief point of entrance of the fungus into the cane seems to be the base of the stalk. Some cases have been observed which suggested the possibility that the disease might begin at an infected root, but in every case the zones could be connected with others proceeding from the base of the stalk. Figure 4, A shows concentric rings formed around a root

FIG. 6. Photograph of the cross section of four stalks of sugar cane infected with zonate foot rot. A shows a part of the stalk infected and a part uninfected. Note the sharp dividing line between the diseased and healthy pith.

entrance into the stalk. The cortex of the root was decayed but the central cylinder appeared to be little affected.

The disease has been traced in its course from the main primary stalk through the point of attachment into the secondary buds, from these through the point of attachment into the tertiary buds and into the shoots of the fourth rank. In every case the destruction was complete: the entire cepa or stool was dead. The zonate markings stay intact and continue through the woody underground parts of the stalks as well as through the internodes. Figure 8 is a diagrammatic longitudinal section showing the relation of the successive branches of the underground stems and the path of spread of the disease.

The infection starts from the infected ratoon or stubble cane as shown in figure 8, 1, and proceeds in turn into stalks number 2, 3, and 4. In many cases observed, the disease had spread to every shoot, both young and old, and the entire stools eventually died. In other instances a stool was found partially infected, but every indication pointed to complete destruction of the hill in time.

### COMPARISON WITH SIMILAR ROOT AND STALK ROTS OF SUGAR CANE

In the root rot of sugar cane we have an infection confined to the roots of the plants in the early stages of the disease. Even plants badly stunted and with very short, stubby roots of one or two centimeters in length often show no signs of stalk rot, though the leaves may be wilting from lack of moisture. In the zonate foot rot the roots of the plant grow vigorously until the upward conduction of the sap is prevented by the dry

FIG. 7. Photograph of young shoots showing progress of zonate rot from older to younger tillers.

rot of the stalk base. In some cases the cortex of a root appears to be infected (Fig. 4, A), but in such cases it has not been determined whether the disease appeared first in the stalk and spread to the root or in the root first. From figure 4 it would seem that the disease had spread from the stalk to the root.

Both root rot and zonate foot rot usually appear in localized spots in the fields, but while root rot is largely confined to areas in which there is some conspicuous cultural defect, such as lack of drainage, etc., the zonate rot appears in vigorously growing cane under good cultural conditions.

In Cuba, as well as in other cane-growing countries, a root and basal stalk rot occurs which follows injury to cane weakened by other agencies, as, for example, high concentrations of injurious salts, soil deficiencies, drought, water-logged soils, and attacks of white grubs, root mealybugs, and wireworms. This is the type most in evidence in the Cuban cane fields. The final rotting is done largely by *Melanconium sacchari*, in some cases *M. ilian*, and some other fungi which have not fruited as yet in cultures. This is the type of trouble generally referred to locally as root disease. A number of experiments have been carried out with the above mentioned fungi, and with finely chopped diseased roots and stalk bases to determine whether these agencies are primarily responsible for the incidence of the disease. Experiments were carried out in both sterilized

FIG. 8. Diagrammatic cross section of a portion of a stool of sugar cane showing the progress of the zonate foot rot. The disease enters the primary shoot (1) from the old infected stubble and progresses through shoots of 2nd, 3rd and 4th rank.

and unsterilized soil taken from diseased areas. No primary infection was secured. It is quite evident that previous weakening is necessary before cane plants succumb to this type of disease. The organisms seem to be semi-parasitic and play a secondary role in the injury of the plant.

In such diseased plants there are cylinders of infected bundle fibers extending through several internodes of the stalks. The plants attacked are stunted in their growth, have no marked zones at the base, but begin wilting and drying long before they finally die. The rot is not dry or woody, as it is in the case of the zonate rot.

Upon seeing cane affected with this zonate rot, Dr. G. Wilbrink, of Java, remarked to the writer that a similar disease is called Stengelbrand,

or stalk burning, in that country. This Javan disease is usually confined to sporadic cases in the young cane, but may become epidemic in a field. In affected plants the young spots on the stalks are a watery red color. The cells in these areas are all dead, the parenchyma dying first and later the sclerenchyma of the vascular strands. A marked difference in the Stengelbrand of Java and the zonate foot rot is the absence of zonated markings in the former. Also there are brown leaf spots associated with the Javan disease by which the affected plants may always be identified. No such spots have been observed in connection with the zonated rot.

### A POSSIBLE CAUSE OF THE DISEASE

While making a periodic inspection in some fields in which there was zonate foot rot in the cane, plants were found with brackets of a Fomes

FIG. 9. Cane stalk affected with zonate foot rot, with the bracket of a species of *Fomes* attached.

species at the base. Longitudinal sections of these stalks showed the characteristic zoning at the ground surface and the fungous strands throughout the diseased portions of the stalks. The brackets were firmly attached to the stalks and so intimately associated with the zonated areas as strongly to suggest that this fungus was the probable causative organism. Figure 9 is a photograph of a portion of an affected stalk upon which has developed a bracket of this *Fomes*.

Specimens of such canes were submitted to Dr. J. R. Weir for identification, but the fungus was sterile. Dr. Weir states "the fungus is a species of *Fomes* with structure peculiar to a group typically represented by *F. pachyphloeus* Pat. and *F. melanodermus* Pat."

Figure 10 shows the brown fungous strands (A) throughout the stalk and the intimate association of the *Fomes* with the zonated portion of the cane stalk (B).

Many canes have been found which show only the zonate foot rot but no case has been found of a cane attacked by the *Fomes* which did not also show the zonated condition.

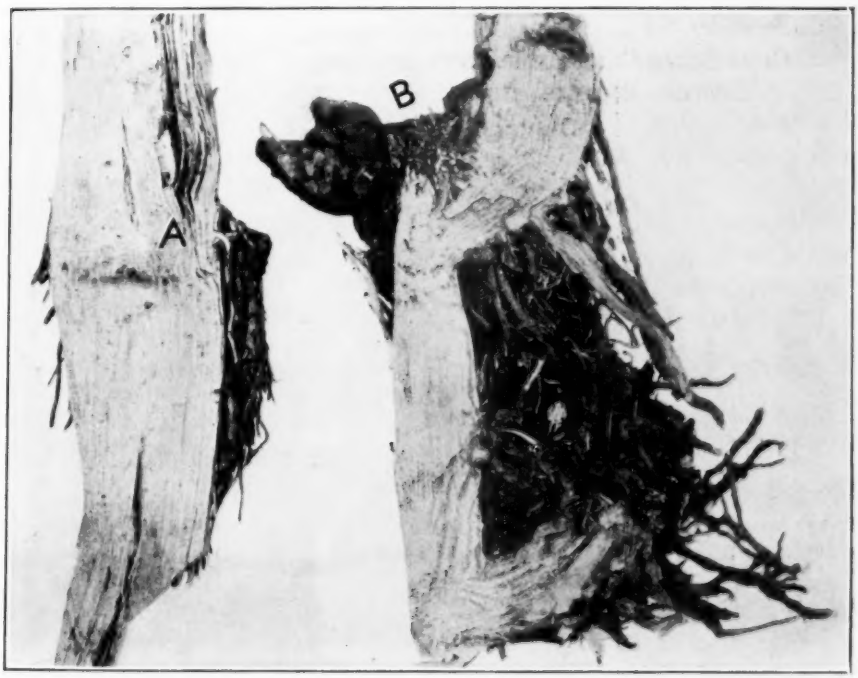



FIG. 10. Longitudinal section of two cane stalks affected with zonate foot rot upon which the Fomes species has developed. A. Brown strands of the *Fomes* throughout the cane stalk. B. *Fomes* intimately associated with the zonated parts of the stalk.

It seems quite possible that species of *Fomes* attacks some tree species in the forests, but neither its exact identification nor its host relationships have been determined. As pointed out before, the zonate foot rot of cane has been found only in fields where the cane had been planted in "monte" land, that is, immediately after the cutting of the forests.

SUMMARY

1. A description is given of a zonate foot rot of sugar cane which occurs in two widely separated localities in Cuba.

2. There is no indication that the disease is limited to certain types of soil, for it has been found on a very heavy black soil with a clay subsoil and on a porous red soil.

3. The disease is characterized by a zonate dry rot at the base of the stalk. Alternating bands of gray and reddish tissue are conspicuous above each node.

4. Fruiting bodies of a species of *Fomes* are often associated with the zonate rot. The species has not been identified, nor its host relationships determined.

TROPICAL PLANT RESEARCH FOUNDATION,
CUBA SUGAR CLUB EXPERIMENT STATION,
CENTRAL BARAGUÁ, CUBA.

# NATURE OF RESISTANCE OF BERBERIS SPP. TO PUCCINIA GRAMINIS[1]

LEONARD W. MELANDER[2] AND J. H. CRAIGIE[3]

## INTRODUCTION

It has been shown that the resistance of wheat varieties to the uredinial stage of *Puccinia graminis tritici* (Pers.) Erikss. and Henn. may be due either to physiological or morphological causes. Furthermore, it has been shown that seedling plants may be susceptible to certain physiologic forms of the rust organism, whereas older plants of the same variety may be resistant (10). It also is known that the sporidial germ tubes of *P. graminis* mechanically force their way directly through the epidermis of the aecial host, and that young leaves of *Berberis vulgaris* L. are susceptible, while the older ones are resistant. This suggests the possibility that resistance may be due to the inability of the fungus to penetrate the epidermis of older leaves. Not only that, but it often has been observed that tough-leaved species of *Berberis* and *Odostemon* seem to be more resistant than tender-leaved species. It seemed possible, therefore, that the resistance of varieties of *Berberis* and *Odostemon* might be due to morphological differences. Therefore an investigation was undertaken to ascertain the following: (a) Can germ tubes of sporidia penetrate the cuticle of resistant varieties? (b) Is there a definite correlation between resistance to puncture and resistance to rust? (c) Is there a correlation between resistance to puncture and thickness of the cuticle or of the epidermal walls?

[1] Cooperative investigation of the Office of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and the Section of Plant Pathology, Department of Agriculture, University of Minnesota. Published with the approval of the Director of the Minnesota Agricultural Experiment Station, as paper No. 636 of the Journal Series.

The writers wish to express their appreciation to Dr. E. C. Stakman for his assistance throughout the investigation, to Mr. R. U. Cotter for his assistance when readings on the Jolly balance were taken, and to Dr. M. N. Levine for his assistance in organizing these data.

[2] Associate Pathologist, Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

[3] Formerly Agent, Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and Assistant Plant Pathologist, University of Minnesota.

HISTORICAL SUMMARY

It is well known that many plant pathogenes enter host plants by direct mechanical penetration of the epidermis. Brown (2), in 1916, found that the germ tubes of *Botrytis cinera* Pers. were unable to affect chemically the cuticle of the host. Blackman and Welsford (1), in the same year, found that the piercing of the cuticle of *Vicia faba* by the germ tube of *Botrytis cinerea* Pers. was " . . . due solely to mechanical pressure exerted by the germ tube as a whole or by the special outgrowth from it." In 1919 Dey (4) found that *Colletotrichum lindemuthianum* (Sacc. and Mag.) Bri. and Cav. formed thick-walled dark-colored appressoria, which became firmly attached to the host plant by a mucilaginous envelope. A peg-like infection hypha grew out from the appressorium and penetrated the cuticular layer by mechanical pressure. Leach (8) observed a similar phenomenon and showed that it took the hyphae longer to penetrate the cell walls of resistant than of susceptible varieties.

It has been known for some time that the germ tubes from sporidia of *Puccinia graminis* Pers. penetrate the cuticle of *Berberis* spp. directly, regardless of natural openings. Waterhouse (13) demonstrated that the penetration is accomplished by mechanical pressure and not by the dissolving action of enzymes.

In 1892 Cobb (3) suggested that resistance of certain Australian wheats to stem rust was correlated with the thickness and tensile strength of cuticle. Later Ward (12) concluded that morphological peculiarities had little effect on the resistance of a host. However, Hawkins and Harvey (5) showed that the resistance of the McCormick variety of potato to infection by *Pythium debaryanum* Hesse was correlated with resistance to mechanical puncture; and Hawkins and Sando (6) showed that cooling the fruit of strawberries, blackberries, black and red raspberries, and cherries made the epidermis more resistant to mechanical puncture. Valleau (11) found that varieties of plums having a thick, tough skin were more resistant to brown rot than thinner-skinned varieties. Willaman *et al* (14) also concluded that the toughness of the skin and the firmness of the flesh of plums are factors in resistance to brown rot. According to Hursh (7), varieties of wheat characterized by abundant sclerenchyma are likely to be injured less by stem rust because this tissue mechanically limits the spread of mycelium within the host. Melhus *et al* (9) suggested that the marked difference in the susceptibility of young and old leaves of the barberry might be due to the thickness of cuticle and epidermis, considering that infection is accomplished by direct penetration of the tissues and not through invasion of stomata.

## MATERIALS AND METHODS

To determine whether the mycelium of *Puccinia graminis* actually enters immune plants, leaves of *Berberis thunbergii* D. C. were inoculated and incubated 4, 5, 6, 7, and 13 days. They then were killed, embedded in paraffin, sectioned, and stained with Fleming's triple stain. They next were examined to ascertain whether the rust mycelium had entered. Observations also were made on fresh leaves of *B. thunbergii* which had been inoculated with sporidia of *P. graminis* Pers.

For determining the thickness of the external walls of the epidermal cells, fresh leaves were taken from plants of various species of *Berberis* growing in the greenhouse. Hand-sections of these leaves were made and measurements taken by means of a filar micrometer. In several cases, fresh leaves of the desired age were not available, so the measurements were made on leaves previously mounted in paraffin. All measurements were made at approximately the middle of the cell wall, thus avoiding the thickenings at the corners of the cells. Leaves of three ages were chosen: 2 to 3 days; 5 to 6 days; and 16 to 20 days, designated in table 1 as mature. Measurements for the two-day-old and three-day-old leaves were tabulated separately at first; but, as they were almost identical, there was no necessity of keeping them separate. Readings for leaves 5 and 6 days old were grouped together for the same reason.

Whenever practicable, readings for any particular age were taken from leaves of several plants of the same species. This could not always be done, as in the case of *B. pruinosa* and *B. lycium*, in which the readings had to be made from leaves of one plant. In general, 20 readings were taken for each leaf of a given age and the averages were based on 100 readings. In some cases, many more readings were made; but the averages, as given in table 1, were not affected thereby. Measurements also were made of the cell walls on the lower surface of the leaves, but, as they usually corresponded very closely with those on the upper side of the leaves, it is not necessary to consider them further.

The thickness of the cuticle as such was not measured. It was so thin in very young leaves, if present at all, that it could not be measured. In the older leaves it was included as part of the cell wall.

Ten-day-old leaves of *B. vulgaris* were sectioned in order to compare the thickness of the cell walls with that of the younger leaves of other species.

A modified Jolly balance (Fig. 4), fitted with a specially ground, round-pointed phonograph needle, was used to measure the pressure required to puncture the cuticle. The approximate diameter of this needle was only 25 microns, and a binocular magnifier was required to see when it had pene-

trated the cuticle. One centimeter on the vernier was equal to a pressure of 518 milligrams. To make conditions as uniform as possible, the puncturing always was done early in the afternoon of sunny days. To insure uniform leaf turgor, plants were maintained in moist soil. As a rule, only five measurements were possible for each leaf because the leaves soon wilted after being cut from the plant and the resistance of wilted leaves to puncture was different from that of turgid leaves. Twenty-five measurements were made of leaves of each species at each age. The ages of the leaves used were 1, 2, 3, 4, 7, 10, and 14 days. The following is a list of the species of *Berberis* and *Odostemon* which were studied:

| Susceptible | Resistant | Immune |
|---|---|---|
| *B. aristata* D. C. | *B. brachypoda* Maxim. | *B. thunbergii* D. C. |
| *B. buxifolia* Lam. | *B. chinensis* Poiret | *O. repens* (Lindl.) Rydb. |
| *B. canadensis* Mill. | *B. lycium* Royle | |
| *B. dictyophylla* Franch | *B. pruinosa* Franch | |
| *B. leichlinii* | *O. aquifolium* (Pursh) Rydb. | |
| *B. vulgaris* Linn. | | |
| *O. swaseyi* (Buckl.) Rydb. | | |

## RESULTS

A few preliminary attempts made to determine whether the sporidial germ tube of *Puccinia graminis* actually enters the immune *B. thunbergii* yielded no evidence that the parasite penetrates the outer epidermal wall. However, these studies were not extensive enough to justify final conclusions.

*Thickness of outer walls of epidermal cells.* Outer walls of the epidermal cells of susceptible varieties were found to be thinner than those of resistant or immune varieties, with the exception of *B. pruinosa* (Table 1). The epidermal cells of this species are quite small, scarcely more than one-third the width of the cells of many of the other species, and have very heavy perpendicular walls, much thickened at the angles. The result is that the central portion of the external wall of the epidermis (the portion for which the measurement is given in table 1) is very much thinner than either of the end portions. Consequently the mean thickness of the cell wall would be almost twice as great as the thickness given in table 1. The walls of two-to-three-day-old leaves of the immune *B. thunbergii* are almost twice as thick as those of the susceptible *B. dictyophylla*, and those of the immune *Odostemon repens* more than twice as thick as those of *B. dictyophylla* and *B. canadensis*, both of which are susceptible.

It will be noted (Table 1) that the thickness of the epidermal walls increases with age and that this increase is more rapid in resistant than in susceptible varieties. Therefore the period of susceptibility of

resistant varieties probably is shorter than that of susceptible varieties. The epidermal walls of the immune *O. repens* are very thick and are covered with a waxy exudate even when the leaves are very young.

TABLE 1.—*Average thickness of the external walls of epidermal cells of the upper surface of leaves of various species of Berberis*

| Species | Age of leaves | | |
|---|---|---|---|
| | 2 and 3 days | 5 and 6 days | Mature |
| | Thickness in microns | | |
| Susceptible | | | |
| *Berberis canadensis* | 0.88 | 0.93 | 1.29 |
| *B. dictyophylla* | 0.82 | 1.23 | 1.80 |
| *B. vulgaris* | 1.10 | 1.18 | 1.87 |
| Resistant | | | |
| *B. brachypoda* | 1.43 | 2.09 | 2.56 |
| *B. lycium* | 1.23 | 2.86 | 3.41 |
| *B. pruinosa* | 1.16 | 1.46 | 2.20 |
| *Odostemon aquifolium* | 1.30[a] | ..... | 3.19[a] |
| Immune | | | |
| *B. thunbergii* | 1.57 | 1.62 | 2.44 |
| *O. repens* | 1.75[a] | ..... | 3.01 |

[a] Measurements from killed material mounted in paraffin.

But the leaves of susceptible species also become practically immune with age. The results obtained in these studies show that this may be due, at least partly, to the thickening of the epidermal cell wall. Ten-day-old leaves of *B. vulgaris* (not included in table 1) have an average cell wall thickness of 1.52 microns, which is slightly less than that of the two- and three-day-old leaves of *B. thunbergii*. Leaves of *B. vulgaris* ten days old are fairly resistant to *P. graminis*. This would indicate a close correlation between resistance and the thickness of the outer epidermal wall. The thickness of the wall, however, does not seem to be the only factor, as young leaves of *B. brachypoda* have comparatively thick-walled epidermal cells but become infected readily, forming numerous pycnia and but few aecia. Obviously the germ tubes have entered the leaves, but some condition inside the leaf prevents normal development.

*Resistance to puncture.* The data on the resistance to puncture of leaves of various species of *Berberis* and *Odostemon* are given in tables 2, 3, 4, 5, 6, 7, 8 and 9. If the resistance of various species of *Berberis* is due to the toughness of the epidermal walls, no species would be more resistant to the rust parasite than the most tender portion of the outer cell walls.

TABLE 2.—*Variations in and constants of the resistance to puncture of the outer walls of epidermal cells of one-day-old leaves of various species of Berberis and Odostemon, as determined by a modified Jolly balance*

| Variety | Resistance to puncture: Class centers in milligrams | | | | | | | | | Constants | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 200 | 250 | 300 | 350 | 400 | 450 | 500 | 550 | 600 | Range | Mean | Standard deviation | Coefficient of variability |
| Susceptible | | | | | | | | | | | | | |
| *B. aristata* | 1 | 1 | 10 | 10 | 2 | 1 | | | | 207.2 — 440.3 | 328 ± 6.62 | 49.0 ± 4.67 | 14.94 ± 1.43 |
| *B. buxifolia* | | 3 | 5 | 12 | 5 | | | | | 233.1 — 388.5 | 338 ± 6.11 | 45.3 ± 4.32 | 13.40 ± 1.28 |
| *B. canadensis* | | 2 | 8 | 11 | 4 | | | | | 259.0 — 388.5 | 334 ± 5.64 | 41.8 ± 3.99 | 12.51 ± 1.19 |
| *B. dictyophylla* | | 2 | 14 | 7 | 2 | | | | | 259.0 — 388.5 | 318 ± 5.00 | 37.1 ± 3.54 | 11.67 ± 1.11 |
| *B. leichlinii* | | 2 | 4 | 9 | 8 | 2 | | | | 259.0 — 466.2 | 358 ± 7.06 | 52.3 ± 4.99 | 14.61 ± 1.39 |
| *B. swaseyi* | | 1 | 7 | 8 | 8 | 1 | | | | 259.0 — 440.3 | 352 ± 6.46 | 47.9 ± 4.57 | 13.61 ± 1.30 |
| *B. vulgaris* | | 2 | 13 | 8 | 2 | | | | | 233.1 — 388.5 | 320 ± 5.05 | 37.4 ± 3.57 | 11.69 ± 1.12 |
| Resistant | | | | | | | | | | | | | |
| *B. brachypoda* | | 5 | 10 | 5 | 4 | 1 | | | | 233.1 — 466.2 | 322 ± 7.42 | 55.0 ± 5.24 | 16.77 ± 1.60 |
| *B. chinensis* | | | | 9 | 8 | 7 | 1 | | | 336.7 — 492.1 | 400 ± 6.03 | 44.7 ± 4.26 | 11.18 ± 1.07 |
| *B. lycium* | | | 3 | 9 | 9 | | 2 | 2 | | 310.8 — 569.8 | 390 ± 9.15 | 67.8 ± 6.47 | 17.38 ± 1.66 |
| *B. pruinosa* | | 1 | | 8 | 10 | 5 | 1 | | | 290.0 — 492.4 | 392 ± 6.79 | 50.3 ± 4.80 | 12.83 ± 1.22 |
| *O. aquifolium* | | 1 | 5 | 15 | 4 | | | | | 259.0 — 414.4 | 344 ± 4.80 | 35.6 ± 3.40 | 10.35 ± 0.99 |
| Immune | | | | | | | | | | | | | |
| *B. thunbergii* | | | | | | 4 | 10 | 6 | 5 | 440.3 — 595.7 | 524 ± 6.64 | 49.2 ± 4.69 | 9.39 ± 0.90 |
| *O. repens* | | 3 | 10 | 6 | 6 | | | | | 233.1 — 414.4 | 330 ± 6.61 | 49.0 ± 4.67 | 14.85 ± 1.42 |

TABLE 3.—*Variations in and constants of the resistance to puncture of the outer walls of epidermal cells of two-day-old leaves of various species of Berberis and Odostemon, as determined by a modified Jolly balance*

| Variety | Resistance to puncture: Class centers in milligrams | | | | | | | | | | | Constants | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 200 | 250 | 300 | 350 | 400 | 450 | 500 | 550 | 600 | 650 | 700 | Range | Mean | Standard deviation | Coefficient of variability |
| Susceptible | | | | | | | | | | | | | | | |
| *B. aristata* | | | 1 | 9 | 8 | 7 | | | | | | 310.8 — 466.2 | 392 ± 5.94 | 44.0 ± 4.20 | 11.22 ± 1.07 |
| *B. buxifolia* | | | | 2 | 16 | 7 | | | | | | 336.7 — 466.2 | 410 ± 3.82 | 28.3 ± 2.70 | 6.90 ± 0.66 |
| *B. canadensis* | | 1 | 5 | 12 | 6 | 1 | | | | | | 259.0 — 440.3 | 352 ± 5.87 | 43.5 ± 4.15 | 12.36 ± 1.18 |
| *B. dictyophylla* | | 1 | 3 | 11 | 7 | 3 | | | | | | 259.0 — 466.2 | 366 ± 6.20 | 46.0 ± 4.38 | 12.77 ± 1.21 |
| *B. leichlinii* | | 1 | 7 | 8 | 6 | 2 | 1 | | | | | 259.0 — 492.1 | 358 ± 7.80 | 57.8 ± 5.51 | 16.15 ± 1.54 |
| *B. vulgaris* | | | 5 | 10 | 5 | 5 | | | | | | 284.9 — 492.1 | 370 ± 6.88 | 51.0 ± 4.86 | 13.78 ± 1.31 |
| *O. swaseyi* | | | 4 | 8 | 8 | 5 | | | | | | 310.8 — 466.2 | 378 ± 6.64 | 49.2 ± 4.69 | 13.02 ± 1.24 |
| Resistant | | | | | | | | | | | | | | | |
| *B. brachypoda* | | | 8 | 17 | | | | | | | | 284.9 — 362.6 | 334 ± 3.14 | 23.3 ± 2.22 | 6.98 ± 0.67 |
| *B. chinensis* | | | | | 6 | 5 | 7 | 6 | 1 | | | 388.5 — 595.7 | 482 ± 8.02 | 59.5 ± 5.66 | 12.34 ± 1.18 |
| *B. lycium* | | | | | 6 | 11 | 6 | 2 | | | | 388.5 — 569.8 | 458 ± 5.94 | 44.0 ± 4.20 | 9.61 ± 0.92 |
| *B. pruinosa* | | | | 4 | 6 | 5 | 9 | 1 | | | | 569.8 — 336.7 | 444 ± 7.95 | 58.9 ± 5.62 | 13.27 ± 1.27 |
| *O. aquifolium* | | | 2 | 6 | 12 | 3 | 2 | | | | | 310.8 — 518.0 | 394 ± 6.69 | 49.6 ± 4.73 | 12.59 ± 1.20 |
| Immune | | | | | | | | | | | | | | | |
| *B. thunbergii* | | | | | | | 1 | 6 | 11 | 6 | 1 | 518.0 — 699.3 | 600 ± 6.03 | 44.7 ± 4.26 | 7.45 ± 0.71 |
| *O. repens* | | | | 9 | 10 | 5 | 1 | | | | | 336.7 — 492.1 | 396 ± 5.69 | 42.2 ± 4.03 | 10.66 ± 1.02 |

TABLE 4.—*Variations in and constants of the resistance to puncture of the outer walls of epidermal cells of three-day-old leaves of various species of Berberis and Odostemon, as determined by a modified Jolly balance*

| Variety | Resistance to puncture | | | | | | | | | | | | | Constants | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Class centers in milligrams | | | | | | | | | | | | | Range | Mean | Standard deviation | Coefficient of variability |
| | 250 | 300 | 350 | 400 | 450 | 500 | 550 | 600 | 650 | 700 | 750 | 800 | 850 | | | | |
| Susceptible | | | | | | | | | | | | | | | | | |
| *B. aristata* | | 1 | 2 | 3 | 9 | 7 | 3 | | | | | | | 318.8 — 569.8 | 456 ± 8.39 | 62.2 ± 5.93 | 13.64 ± 1.30 |
| *B. buxifolia* | 2 | 4 | 2 | 8 | 9 | | | | | | | | | 233.1 — 466.2 | 386 ± 8.84 | 65.6 ± 6.26 | 16.99 ± 1.62 |
| *B. canadensis* | | | 2 | 7 | 9 | 7 | | | | | | | | 362.6 — 492.1 | 442 ± 6.23 | 46.2 ± 4.41 | 10.45 ± 1.00 |
| *B. dictyophylla* | | | 2 | 2 | 5 | 6 | 4 | 3 | 3 | | | | | 362.6 — 673.4 | 508 ± 11.53 | 85.5 ± 8.15 | 16.83 ± 1.61 |
| *B. lcichlinii* | | | | 3 | 12 | 6 | 3 | 1 | | | | | | 414.4 — 595.7 | 474 ± 6.64 | 49.2 ± 4.69 | 10.38 ± 0.99 |
| *B. vulgaris* | 1 | 3 | 6 | 3 | 5 | 6 | 1 | | | | | | | 259.0 — 543.9 | 410 ± 10.79 | 80.0 ± 7.63 | 19.51 ± 1.86 |
| *O. swaseyi* | | 1 | 3 | 10 | 9 | 1 | 1 | | | | | | | 284.9 — 543.9 | 418 ± 6.85 | 50.8 ± 4.85 | 12.15 ± 1.16 |
| Resistant | | | | | | | | | | | | | | | | | |
| *B. brachypoda* | | 6 | 12 | 6 | 1 | | | | | | | | | 284.9 — 466.2 | 354 ± 5.37 | 39.8 ± 3.80 | 11.24 ± 1.07 |
| *B. chinensis* | | 1 | 3 | 3 | 8 | 5 | 3 | 1 | 1 | | | | | 310.8 — 673.4 | 462 ± 10.85 | 80.4 ± 7.67 | 17.40 ± 1.66 |
| *B. lycium* | | | | | 2 | 7 | 6 | 4 | 2 | 2 | 2 | | | 466.2 — 751.1 | 572 ± 11.46 | 85.0 ± 8.11 | 14.86 ± 1.42 |
| *O. aquifolium* | | | | 3 | 7 | 9 | 2 | 4 | | | | | | 414.4 — 595.7 | 494 ± 8.16 | 60.5 ± 5.77 | 12.25 ± 1.17 |
| Immune | | | | | | | | | | | | | | | | | |
| *B. thunbergii* | | | | | 3 | 12 | 6 | 2 | 2 | | | | | 440.3 — 647.5 | 526 ± 7.16 | 53.1 ± 5.07 | 10.10 ± 0.96 |
| *O. repens* | | | | | | | | 5 | 5 | 6 | 6 | 2 | 1 | 595.7 — 854.7 | 696 ± 9.34 | 69.2 ± 6.60 | 9.94 ± 0.95 |

TABLE 5.—*Variations in and constants of the resistance to puncture of the outer walls of epidermal cells of four-day-old leaves of various species of Berberis and Odostemon, as determined by a modified Jolly balance*

| Variety | Resistance to puncture: Class centers in milligrams | | | | | | | | | | | Constants | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 350 | 400 | 450 | 500 | 550 | 600 | 650 | 700 | 750 | 800 | 850 | Range | Mean | Standard deviation | Coefficient of variability |
| Susceptible | | | | | | | | | | | | | | | |
| *B. aristata* | 1 | 9 | 6 | 2 | 5 | | 2 | | | | | 362.6 — 647.5 | 468 ± 10.77 | 79.8 ± 7.61 | 17.05 ± 1.63 |
| *B. buxifolia* | 1 | 5 | 13 | 6 | | | | | | | | 362.6 — 518.0 | 448 ± 5.22 | 38.7 ± 3.69 | 8.64 ± 0.82 |
| *B. canadensis* | | 4 | 12 | 5 | 2 | 1 | 1 | | | | | 388.5 — 647.5 | 474 ± 8.12 | 60.2 ± 5.74 | 12.70 ± 1.21 |
| *B. dictyophylla* | | | | | 1 | 5 | 7 | 11 | 1 | | | 543.9 — 751.1 | 662 ± 6.41 | 47.5 ± 4.53 | 7.18 ± 0.68 |
| *B. leichlinii* | | | 1 | 5 | 6 | 5 | 2 | 5 | 1 | | | 466.2 — 751.1 | 592 ± 10.91 | 80.9 ± 7.72 | 13.67 ± 1.30 |
| *B. vulgaris* | 1 | 10 | 9 | 3 | 1 | 1 | | | | | | 362.6 — 595.7 | 442 ± 7.31 | 54.2 ± 5.17 | 12.26 ± 1.17 |
| *O. swaseyi* | | | 5 | 5 | 5 | 3 | 5 | 2 | | | | 440.3 — 699.3 | 558 ± 10.91 | 80.9 ± 7.72 | 14.50 ± 1.38 |
| Resistant | | | | | | | | | | | | | | | |
| *B. bracypoda* | 4 | 7 | 6 | 6 | 2 | | | | | | | 336.7 — 569.8 | 440 ± 8.09 | 60.0 ± 5.72 | 13.64 ± 1.30 |
| *B. chinensis* | | | 1 | 2 | 6 | 5 | 10 | 1 | | | | 466.2 — 699.3 | 598 ± 8.20 | 60.8 ± 5.80 | 10.17 ± 0.97 |
| *B. lycium* | | | 3 | 3 | 6 | 11 | 2 | | | | | 440.3 — 673.4 | 562 ± 7.70 | 57.1 ± 5.45 | 10.16 ± 0.97 |
| *O. aquifolium* | | | 1 | 4 | 11 | 2 | 3 | 4 | | | | 466.2 — 725.2 | 578 ± 9.55 | 70.8 ± 6.75 | 12.25 ± 1.17 |
| Immune | | | | | | | | | | | | | | | |
| *B. thunbergii* | | | | 3 | 7 | 13 | 2 | | | | | 466.2 — 647.5 | 578 ± 5.42 | 40.2 ± 3.83 | 6.96 ± 6.64 |
| *O. repens* | | | | | 3 | 1 | 3 | 7 | 3 | 5 | 3 | 569.8 — 854.7 | 716 ± 12.17 | 90.2 ± 8.61 | 12.59 ± 1.20 |

TABLE 6.—*Variations in and constants of the resistance to puncture of the outer walls of epidermal cells of seven-day-old leaves of various species of Berberis and Odostemon, as determined by a modified Jolly balance*

| Variety | Resistance to puncture: Class centers in milligrams | | | | | | | | Constants | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 300 | 400 | 500 | 600 | 700 | 800 | 900 | 1000 | Range | Mean | Standard deviation | Coefficient of variability |
| Susceptible | | | | | | | | | | | | |
| *B. aristata* | | 1 | 13 | 8 | 3 | | | | 414.4 — 673.4 | 552 ± 10.18 | 75.5 ± 7.20 | 13.68 ± 1.30 |
| *B. buxifolia* | | 1 | 9 | 14 | 1 | | | | 466.2 — 699.3 | 640 ± 8.53 | 63.2 ± 6.03 | 9.88 ± 0.94 |
| *B. canadensis* | | 4 | 11 | | 2 | 5 | 3 | | 466.2 — 1010.1 | 612 ± 23.31 | 172.8 ± 16.48 | 28.23 ± 2.69 |
| *B. dictyophylla* | | 3 | 6 | 2 | 5 | 4 | 5 | | 362.6 — 880.6 | 664 ± 23.18 | 171.8 ± 16.39 | 25.87 ± 2.47 |
| *B. leichlinii* | | | 1 | 13 | 10 | | 1 | | 543.9 — 854.7 | 648 ± 10.18 | 75.5 ± 7.20 | 11.65 ± 1.11 |
| *O. swaseyi* | | | | 3 | 11 | 6 | 5 | | 492.1 — 828.8 | 752 ± 12.72 | 94.3 ± 9.00 | 12.54 ± 1.26 |
| *B. vulgaris* | 1 | 2 | 5 | 3 | 8 | 5 | 1 | | 284.9 — 880.6 | 636 ± 20.13 | 149.2 ± 14.23 | 23.45 ± 2.25 |
| Resistant | | | | | | | | | | | | |
| *B. brachypoda* | | | 4 | 11 | 6 | 4 | | | 466.2 — 828.8 | 640 ± 12.65 | 93.8 ± 8.95 | 14.66 ± 1.40 |
| *B. chinensis* | | 7 | 18 | | | | | | 362.6 — 518.0 | 472 ± 6.06 | 44.9 ± 4.28 | 9.51 ± 0.91 |
| *B. lycium* | | | | 5 | 7 | 11 | 2 | | 569.8 — 880.6 | 740 ± 12.06 | 89.4 ± 8.53 | 12.08 ± 1.15 |
| *O. aquifolium* | | | 3 | 11 | 9 | 1 | 1 | | 518.0 — 880.6 | 644 ± 12.11 | 89.8 ± 8.57 | 13.94 ± 1.33 |
| Immune | | | | | | | | | | | | |
| *B. thunbergii* | | | | 12 | 9 | 4 | | | 569.8 — 802.9 | 668 ± 9.89 | 73.3 ± 6.99 | 10.97 ± 1.05 |
| *O. repens* | | | | 2 | 8 | 9 | 5 | 1 | 647.5 — 958.3 | 780 ± 13.22 | 98.0 ± 9.35 | 12.56 ± 1.20 |

TABLE 7.—*Variations in and constants of the resistance to puncture of the outer walls of epidermal cells of ten-day-old leaves of various species of Berberis and Odostemon, as determined by a modified Jolly balance*

| Variety | Resistance to puncture | | | | | | | | | | Constants | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Class centers in milligrams | | | | | | | | | | Range | Mean | Standard deviation | Coefficient of variability |
| | 400 | 500 | 600 | 700 | 800 | 900 | 1000 | 1100 | 1200 | 1300 | | | | |
| Susceptible | | | | | | | | | | | | | | |
| *B. aristata* | | | 1 | 5 | 12 | 4 | 1 | 2 | | | 647.5 — 1061.9 | 820 ± 15.73 | 116.6 ± 11.12 | 14.22 ± 1.36 |
| *B. buxifolia* | | 8 | 10 | 7 | | | | | | | 492.1 — 725.2 | 596 ± 10.44 | 77.4 ± 7.38 | 12.99 ± 1.24 |
| *B. canadensis* | | | 7 | 4 | 6 | 4 | 2 | 2 | | | 621.6 — 1061.9 | 784 ± 21.14 | 156.7 ± 14.95 | 19.99 ± 1.91 |
| *B. leichlinii* | | 6 | 5 | 7 | 2 | 4 | 1 | | | | 466.2 — 958.3 | 684 ± 20.07 | 148.8 ± 14.19 | 21.75 ± 2.07 |
| *B. vulgaris* | | 5 | 10 | 4 | 4 | 2 | | | | | 518.0 — 880.6 | 652 ± 16.24 | 120.4 ± 11.48 | 18.47 ± 1.76 |
| *O. swaseyi* | | | | 1 | 3 | 6 | 9 | 6 | | | 751.1 — 1139.6 | 964 ± 14.72 | 109.1 ± 10.41 | 11.32 ± 1.08 |
| Resistant | | | | | | | | | | | | | | |
| *B. brachypoda* | | 2 | 9 | 6 | 7 | 1 | | | | | 518.0 — 854.7 | 684 ± 14.11 | 104.6 ± 9.98 | 15.29 ± 1.46 |
| *B. chinensis* | 5 | 17 | 2 | 1 | | | | | | | 414.4 — 673.4 | 496 ± 8.93 | 66.2 ± 6.31 | 13.35 ± 1.27 |
| *B. lycium* | | | 2 | 2 | 14 | 6 | 1 | | | | 647.5 — 958.3 | 808 ± 12.02 | 89.1 ± 8.50 | 11.03 ± 1.05 |
| *O. aquifolium* | | 1 | 1 | 8 | 9 | 5 | 1 | | | | 518.0 — 1010.1 | 776 ± 14.42 | 106.9 ± 10.20 | 13.78 ± 1.31 |
| Immune | | | | | | | | | | | | | | |
| *B. thunbergii* | | | | 4 | 14 | 5 | 2 | | | | 699.3 — 1036.0 | 820 ± 10.79 | 80.0 ± 7.63 | 9.76 ± 0.93 |
| *O. repens* | | | | | 1 | 9 | 10 | 4 | 1 | | 828.8 — 1165.5 | 980 ± 12.06 | 89.4 ± 8.53 | 9.12 ± 0.87 |

TABLE 8.—*Variations in ana constants of the resistance to puncture of the outer walls of epidermal cells of fourteen-day-old leaves of various species of Berberis and Odostemon, as determined by a modified Jolly balance*

| Variety | Resistance to puncture | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Class centers in milligrams | | | | | | | | | | | | | | | | | | | |
| | 400 | 500 | 600 | 700 | 800 | 900 | 1000 | 1100 | 1200 | 1300 | 1400 | 1500 | 1600 | 1700 | 1800 | 1900 | 2000 | 2100 | 2200 | 2300 |
| Susceptible | | | | | | | | | | | | | | | | | | | | |
| *B. aristata* | | | | 1 | 6 | 7 | 9 | 2 | | | | | | | | | | | | |
| *B. buxifolia* | | | | | 4 | 9 | 9 | 2 | 1 | | | | | | | | | | | |
| *B. canadensis* | | | | | 4 | 7 | 2 | 6 | 5 | | 1 | | | | | | | | | |
| *B. leichlinii* | | 1 | 1 | 1 | 1 | 5 | 9 | 5 | 2 | | | | | | | | | | | |
| *B. vulgaris* | | 1 | 9 | 6 | 6 | 3 | | | | | | | | | | | | | | |
| *O. swaseyi* | | | | | | | | 2 | 5 | 4 | 5 | 1 | 3 | 1 | 1 | 1 | 1 | | | 1 |
| Resistant | | | | | | | | | | | | | | | | | | | | |
| *B. brachypoda* | | | | | | 10 | 4 | 8 | 3 | | | | | | | | | | | |
| *B. chinensis* | 4 | 11 | 7 | 3 | | | | | | | | | | | | | | | | |
| *B. lycium* | | | 1 | 4 | 6 | 6 | 7 | 1 | | | | | | | | | | | | |
| *O. aquifolium* | | | | 2 | 11 | 8 | 2 | 2 | | | | | | | | | | | | |
| Immune | | | | | | | | | | | | | | | | | | | | |
| *B. thunbergii* | | | | | | 10 | 12 | 3 | | | | | | | | | | | | |
| *O. repens* | | | | | | 1 | 4 | 10 | 5 | 3 | 1 | | 1 | | | | | | | |

TABLE 8.—(*Continued*)

| Variety | Constants | | | |
|---|---|---|---|---|
| | Range | Mean | Standard deviation | Coefficient of variability |
| Susceptible | | | | |
| *B. aristata* | 751.1–1061.9 | 920 ± 13.75 | 101.9 ± 9.72 | 11.08 ± 1.06 |
| *B. buxifolia* | 751.1–1191.4 | 948 ± 13.29 | 98.5 ± 9.40 | 10.39 ± 0.99 |
| *B. canadensis* | 828.8–1372.7 | 1020 ± 21.58 | 160.0 ± 15.26 | 15.69 ± 1.50 |
| *B. leichlinii* | 518.0–1243.2 | 960 ± 22.25 | 164.9 ± 15.73 | 17.18 ± 1.64 |
| *B. vulgaris* | 466.2– 880.6 | 704 ± 15.01 | 111.3 ± 10.62 | 15.81 ± 1.51 |
| *O. swaseyi* | 1061.9–2305.1 | 1456 ± 39.49 | 292.7 ± 27.92 | 20.10 ± 1.92 |
| Resistant | | | | |
| *B. brachypoda* | 854.7–1243.2 | 1016 ± 14.62 | 108.4 ± 10.34 | 10.67 ± 1.02 |
| *B. chinensis* | 362.6– 673.4 | 536 ± 11.99 | 88.9 ± 8.48 | 16.59 ± 1.58 |
| *B. lycium* | 647.5–1061.9 | 868 ± 16.94 | 125.6 ± 11.98 | 14.47 ± 1.38 |
| *O. aquifolium* | 725.2–1061.9 | 864 ± 13.69 | 101.5 ± 9.68 | 11.75 ± 1.12 |
| Immune | | | | |
| *B. thunbergii* | 880.6–1061.9 | 972 ± 8.96 | 66.4 ± 6.33 | 6.83 ± 0.65 |
| *O. repens* | 932.4–1605.8 | 1152 ± 19.51 | 144.6 ± 13.79 | 12.55 ± 1.20 |

TABLE 9.—*Summary of the mean pressures in milligrams required to penetrate the outer walls of epidermal cells of leaves of various species of Berberis and Odostemon, as determined by a modified Jolly balance*

| Variety | Mean pressure in milligrams | | | | | | |
|---|---|---|---|---|---|---|---|
| | Age of leaves in days | | | | | | |
| | 1 | 2 | 3 | 4 | 7 | 10 | 14 |
| Susceptible | | | | | | | |
| *B. aristata* | 328 ± 6.62 | 392 ± 5.94 | 456 ± 8.39 | 468 ± 10.77 | 552 ± 10.18 | 820 ± 15.73 | 920 ± 13.75 |
| *B. buxifolia* | 338 ± 6.11 | 410 ± 3.82 | 386 ± 8.84 | 448 ± 5.22 | 640 ± 8.53 | 596 ± 10.44 | 948 ± 13.29 |
| *B. canadensis* | 334 ± 5.64 | 352 ± 5.87 | 442 ± 6.23 | 474 ± 8.12 | 612 ± 23.31 | 784 ± 21.14 | 1020 ± 21.58 |
| *B. dictyophylla* | 318 ± 5.00 | 366 ± 6.20 | 508 ± 11.54 | 662 ± 6.41 | 644 ± 23.18 | | |
| *B. leichlinii* | 358 ± 7.06 | 358 ± 7.80 | 474 ± 6.64 | 592 ± 10.91 | 648 ± 10.18 | 684 ± 20.07 | 960 ± 22.25 |
| *O. swayseyi* | 352 ± 6.46 | 378 ± 6.64 | 418 ± 6.85 | 558 ± 9.85 | 752 ± 12.72 | 964 ± 14.72 | 1456 ± 39.49 |
| *B. vulgaris* | 320 ± 5.05 | 370 ± 6.88 | 410 ± 10.79 | 442 ± 10.91 | 636 ± 20.13 | 652 ± 16.24 | 704 ± 15.01 |
| Resistant | | | | | | | |
| *B. brachypoda* | 322 ± 7.42 | 334 ± 3.14 | 354 ± 5.37 | 440 ± 8.09 | 640 ± 12.65 | 684 ± 14.11 | 1016 ± 14.62 |
| *B. chinensis* | 400 ± 6.03 | 482 ± 8.02 | 462 ± 10.85 | 598 ± 8.20 | 472 ± 6.06 | 496 ± 8.93 | 536 ± 11.99 |
| *B. lycium* | 390 ± 9.15 | 458 ± 5.94 | 572 ± 11.46 | 562 ± 7.70 | 740 ± 12.06 | 808 ± 12.02 | 868 ± 16.94 |
| *B. pruinosa* | 392 ± 6.79 | 444 ± 7.95 | | | | | |
| *O. aquifolium* | 344 ± 4.80 | 394 ± 6.69 | 494 ± 8.16 | 578 ± 9.55 | 644 ± 12.11 | 776 ± 14.42 | 864 ± 13.69 |
| Immune | | | | | | | |
| *B. thunbergii* | 524 ± 6.64 | 600 ± 6.03 | 526 ± 7.16 | 578 ± 5.42 | 668 ± 9.89 | 820 ± 10.79 | 972 ± 8.96 |
| *O. repens* | 330 ± 6.61 | 396 ± 5.69 | 696 ± 9.34 | 716 ± 12.17 | 780 ± 13.22 | 980 ± 12.06 | 1152 ± 19.51 |

Young leaves are most easily punctured and are also most susceptible to stem rust. In general, one-day-old leaves of susceptible varieties were found to be less resistant to puncture than those of resistant or immune varieties. The difference between the resistance to puncture of one-day-old leaves of *B. thunbergii* (immune) and *B. vulgaris* (susceptible) is more than 24 times the probable error of the difference. It will be noted that one-day-old leaves of *B. chinensis*, which is definitely known to be quite resistant, has a puncture index intermediate between those of the susceptible and immune varieties. The somewhat resistant *B. brachypoda*, with a puncture index equal to that of susceptible species, may possess some internal resistance. One-day-old leaves of *B. pruinosa* (resistant) have a high mean index of resistance to puncture, but it will be noted that it also has a small minimum and a large maximum (Table 2). The nature of the epidermal cells of this species already has been noted. Owing to the fact that the epidermal cells are small, there are more of the thick perpendicular walls per unit of area to lend support to the horizontal walls when pressure

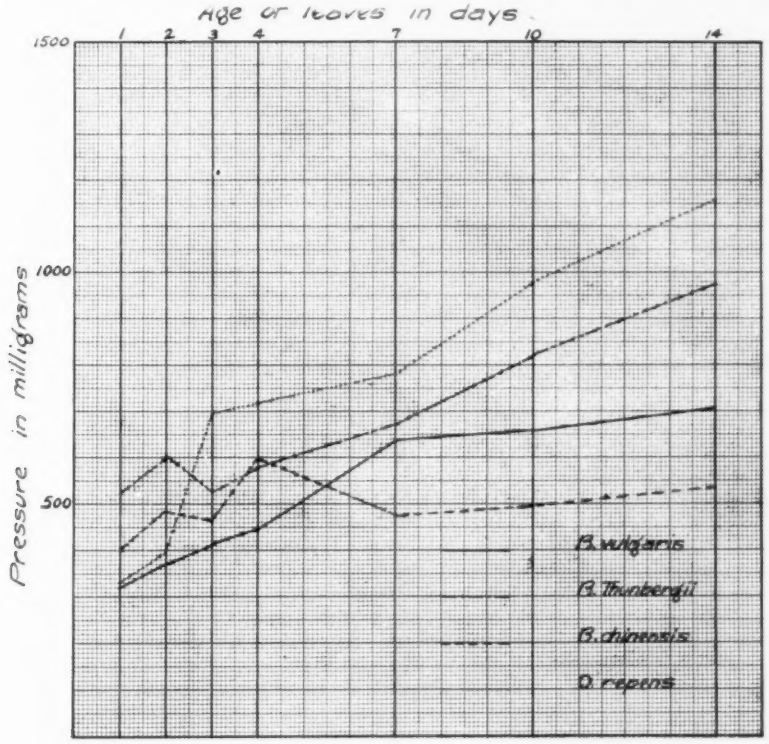



FIG. 1. Variation in resistance to puncture of the outer epidermal wall of leaves of *B. vulgaris*, *B. thunbergii*, *B. chinensis*, and *O. repens*, as measured by a modified Jolly balance.

is exerted upon them, and the thick perpendicular walls possibly may be a factor in preventing the growth of hyphae after entrance has been gained.

It will be noted also that the puncture index for one-day-old leaves of *Odostemon repens* is rather low. It already has been pointed out that the epidermal walls of the young leaves of this species are quite thick. The low resistance of one-day-old leaves to puncture might be explained by the fact that the cuticle of the leaves of this age is merely a waxy exudate which would offer very little resistance to puncture. In this case the pressure required to puncture the epidermal wall would be practically the resistance of the epidermal wall without the cuticle. Starting with the second day, however, the cuticle hardens rapidly and becomes very resistant to puncture (Fig. 1).

Reference already has been made to the rapidity with which the resistance to puncture of some species of *Berberis* and *Odostemon* increases. The resistance to puncture increases at about the same rate as the thickening of the epidermal wall and cuticle, although toughness and not mere

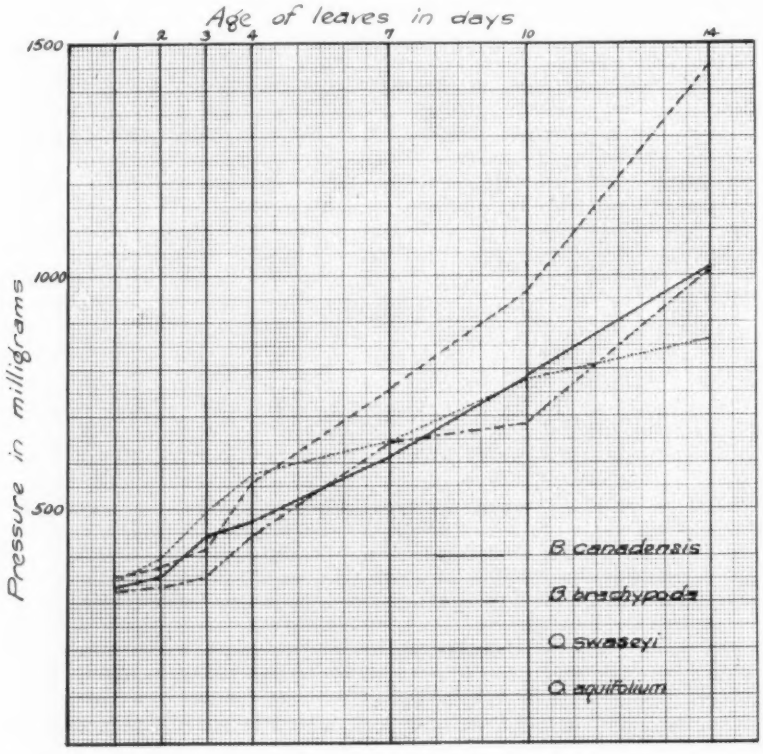



FIG. 2. Variation in resistance to puncture of the outer epidermal wall of leaves of *B. canadensis*, *B. brachypoda*, *O. swaseyi*, and *O. aquifolium*, as measured by a modified Jolly balance.

thickness may also be very important. While the puncture index of one-day-old leaves of *O. repens* is approximately the same as that of leaves of *B. vulgaris* of the same age, the index of three-day-old leaves is 50 per cent greater than that of leaves of *B. vulgaris* of the same age. In fact, the three-day-old leaves of *O. repens* are more resistant to puncture than those of *B. thunbergii*. This would mean that the soft cuticle must have hardened and resistance may be due partly to the rapid hardening of the cuticle. Leaves of *B. vulgaris* increase in toughness with age: seven-day-old leaves are more resistant to puncture than one-day-old leaves of *B. thunbergii*. While the one-day-old leaves of *B. chinensis* are intermediate in their resistance to puncture, they do not seem to increase in toughness like those of many of the other varieties. The tendency of most species to toughen quite rapidly probably explains the tendency of the leaves of susceptible species to become practically immune with age (Figures 1, 2, and 3).

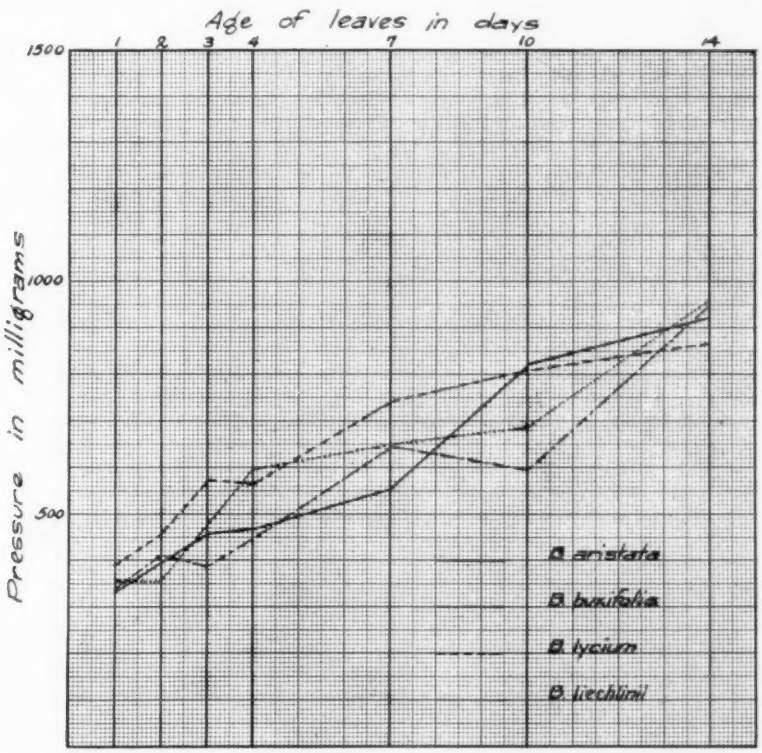



FIG. 3. Variation in resistance to puncture of the outer epidermal wall of leaves of *B. aristata*, *B. buxifolia*, *B. lycium*, and *B. liechlinii*, as measured by a modified Jolly balance.

CONCLUSIONS

The results of the investigation show rather conclusively that barberries may be resistant to *Puccinia graminis* because of morphological characters. It is obvious, however, that this does not necessarily explain the resistance of all varieties. There evidently is also a physiological resistance. The germ tubes are unable to penetrate the epidermal cell walls of some varieties, but can penetrate those of other varieties readily. Subsequent devel-

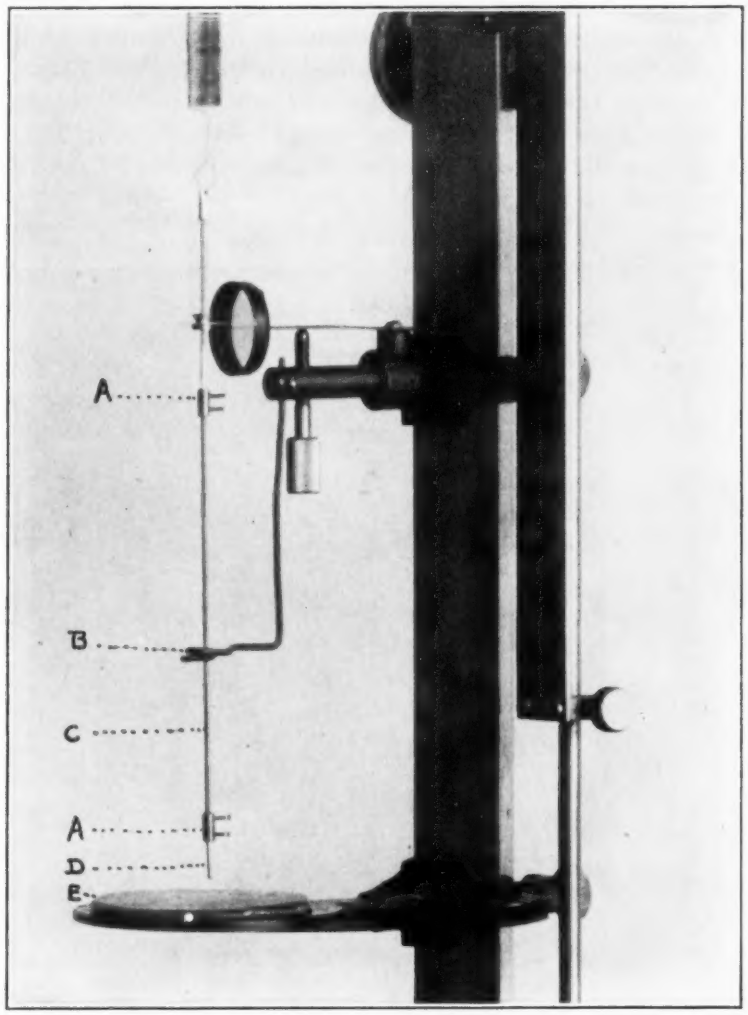



FIG. 4. Modified Jolly balance. A. Coupling. B. Guide. C. Brass rod. D. Phonograph needle with specially ground point. E. Paraffin block.

opment of the organism, however, depends upon the variety concerned. Certainly the germ tubes are able to enter some varieties without being able to develop beyond that point.

Species of *Berberis* very resistant to puncture usually also are resistant to rust. This is well shown by *B. thunbergii* (immune), *B. chinensis* (resistant), and *B. lycium* (resistant). The reason for the inability of the germ tubes to penetrate seems to be threefold. In some varieties the outer walls of the epidermal cells are very thick and resistant to puncture. In other varieties there is a thick cuticle; and in still others the perpendicular walls of the epidermal cells are thick and the epidermal cells themselves are small, an arrangement which probably increases the resistance to puncture.

It has been shown quite clearly that the reason young leaves of barberry often are susceptible but become resistant with age is that the epidermal walls become increasingly resistant to puncture as the leaves grow older. This is shown clearly by *Odostemon repens* and *O. aquifolium*. The outer walls are thick, but easy to puncture. Later, however, they become very resistant to puncture, apparently owing to the production of a waxy exudate which becomes a thick, tough cuticle within about three days.

## SUMMARY

1. The resistance of barberries to stem rust may be due either to morphological or to physiological causes.

2. No indication was found that the sporidial germ tube of *Puccinia graminis* entered an immune host like *Berberis thunbergii*.

3. The leaves of *B. thunbergii* (immune) have much thicker outer walls on the epidermal cells and are more resistant to puncture than those of susceptible species.

4. The difference between the resistance to puncture of one-day-old leaves of *B. thunbergii* (immune) and *B. vulgaris* (susceptible) is more than 24 times the probable error of the difference. One-day-old leaves of *B. chinensis* (resistant) have a puncture index intermediate between those of the susceptible and immune varieties.

5. The resistance of neither *B. brachypoda* nor *B. pruinosa* can be explained on the basis of resistance to puncture.

6. Immunity of *Odostemon repens* probably is partly physiological. However, the cuticle of both this species and *O. aquifolium* (resistant) becomes thick and resistant to puncture within a few days.

7. Those varieties whose leaves are characterized by thin and easily punctured outer walls of the epidermal cells were most susceptible.

8. That leaves of susceptible and resistant varieties become immune from puncture with increasing age can be explained by the thickening of the outer epidermal wall and the accompanying rapid increase in resistance to puncture.

9. The results obtained seem to indicate rather clearly that those species of *Berberis* which are very resistant to puncture usually are resistant to rust also. The converse, however, is not necessarily true. In addition to this morphological resistance, it appears that there also is a physiological resistance. While resistance to puncture, therefore, probably indicates real resistance to the rust organism and possibly may be used as a criterion for resistance, ease of puncture does not necessarily indicate susceptibility.

UNIVERSITY FARM, ST. PAUL, MINNESOTA, AND
OFFICE OF CEREAL CROPS AND DISEASES,
BUREAU OF PLANT INDUSTRY,
WASHINGTON, D. C.

LITERATURE CITED

1. BLACKMAN, V. H., and E. J. WELSFORD. Studies in the physiology of parasitism II. Infection by *Botrytis cinerea*. Ann. Bot. **30**: 389–397. 1916.
2. BROWN, W. Studies in the physiology of parasitism III. On the relation between the "infection drop" and the underlying host tissue. Ann. Bot. **30**: 399–406. 1916.
3. COBB, N. A. Contributions to an economic knowledge of the Australian rusts. Agr. Gaz. New South Wales **3**: 181–212. 1892.
4. DEY, P. K. Studies in the physiology of parasitism V. Infection by *Colletotrichum lindemuthianum*. Ann. Bot. **33**: 305–311. 1919.
5. HAWKINS, LON. A., and RODNEY B. HARVEY. Physiological study of the parasitism of *Pythium debaryanum* Hesse on the potato tuber. Jour. Agr. Res. **18**: 275–297. 1919.
6. ———, and CHARLES E. SANDO. Effect of temperature on the resistance to wounding of certain small fruits and cherries. U. S. Dept. Agr. Bul. 830. 1920.
7. HURSH, C. R. Morphological and physiological studies on the resistance of wheat to *Puccinia graminis tritici* (Pers.) Erik. and Henn. Jour. Agr. Res. **27**: 381–411. 1924.
8. LEACH, J. G. The parasitism of *Colletotrichum lindemuthianum*. Minn. Agr. Exp. Sta. Tech. Bul. 14. 1923.
9. MELHUS, I. E., L. W. DURRELL, and R. S. KIRBY. Relation of the barberry to stem rust in Iowa. Ia. Exp. Sta. Res. Bul. 57. 1920.
10. STAKMAN, E. C., and F. J. PIEMEISEL. Biologic forms of *Puccinia graminis* on cereals and grasses. Jour. Agr. Res. **10**: 429–495. 1917.
11. VALLEAU, W. D. Varietal resistance of plums to brown rot. Jour. Agr. Res. **5**: 365–395. 1915.
12. WARD, H. MARSHALL. Further observations on the brown rust of the Bromes, *Puccinia dispersa* (Eriksson) and its adaptive parasitism. Ann. Bot. **16**: 233–315. 1902.
13. WATERHOUSE, W. L. Studies in the physiology of parasitism VII. Infection of *Berberis vulgaris* by sporidia of *Puccinia graminis*. Ann. Bot. **35**: 557–564. 1921.
14. WILLAMAN, J. J., N. C. PERVIER, and H. O. TRIEBOLD. Biochemistry of plant diseases V. Relation between susceptibility to brown rot in plums and physical and chemical properties. Bot. Gaz. **80**: 121–144. 1925.

# A NEW ALTERNARIA DISEASE OF ONIONS (ALLIUM CEPA L.)

J. A. B. Nolla

This paper deals with an Alternaria disease which appears to be distinct from similar maladies of onions heretofore described. A very severe outbreak of the disease occurred during December, 1924, and January, 1925, in three different places in Porto Rico: in the central part near Barros, in the north near Vega Baja, and in the Experiment Station grounds at Rio Piedras. A study of the pathogene has been made, including its morphology, cultural characters and causal relation to the disease.

## SUSCEPTS

The market onion (*Allium cepa* L.) and the false shallot (*Allium cepa* L., unnamed variety)[2] are the only species known to be affected by this disease.

It appears from field observations that all varieties of onions grown in Porto Rico are equally susceptible. These include the red, yellow, and white varieties of the so-called Bermuda onion. Plants of all ages appear to be susceptible but the disease is apparently most destructive just before bulb formation has started. Of course much depends on the season, and it may be that the injury will be equally great on plants at any age under favorable seasonal conditions.

## THE DISEASE

### *Name*

Since the purple color of the lesions is characteristic of the disease and distinguishes it from other known leaf diseases of the onion, the name "purple leaf spot" is here proposed for it.

[1] Presented to Cornell University in partial fulfillment of the requirements for the degree of Master of Science.

The writer gratefully acknowledges the assistance and suggestions of Dr. Mel. T. Cook, Plant Pathologist, Insular Experiment Station, Porto Rico, under whose direction this work was begun. He is especially indebted to Professor H. H. Whetzel, of the Department of Plant Pathology, Cornell University, under whose direction the work has been completed, for his suggestions and criticisms in the final preparation of the paper.

[2] In Porto Rico and other countries a race of *Allium cepa* with small multiplier bulbs has been called shallot. It does not bear any resemblance to the true shallot. It is here referred to as false shallot as suggested by L. H. Bailey.

HISTORY AND RANGE

Three similar diseases of the onion have been reported. The so-called leaf blight, caused by *Macrosporium parasiticum* Thüm., generally has been regarded as a secondary malady. There is moreover no experimental evidence of an independent causal relation of the fungus involved. The symptoms produced on the suscept following the entrance of the fungus through wounds are distinct from those of the disease in hand. It is distinguished by large dark to brown, sometimes light, spots (14, p. 235), while the disease here studied is characterized by purple lesions.

Another related disease is that produced by *Macrosporium porri* Ellis. The following quotation from Thaxter (15, p. 161) gives a good symptom picture of this malady: The disease "occurs commonly upon seed though less frequently on market onions. . . . The spots are paler than those which characterize *M. parasiticum;* usually more circumscribed and less inclined to inflict injury by the rotting and breaking of the seed stalk at the diseased point." The purple leaf spot causes a distinct rotting of the tissues, as will be pointed out later. Again, the characteristic coloration of the lesions and the fact that it occurs on the foliage of market onions are points which further emphasize its distinctness from the leaf spot caused by *M. porri.*

Ajrekar (1) reported a disease which was threatening the cultivation of onions in Bombay and which he thought probably due to a species of *Macrosporium.* The following year he reported the same disease as a leaf spot and blight, attributing it to *Alternaria sp.* (2). He holds it to be favored by the presence of thrips and states that the fungus is a wound parasite. He finds red varieties less susceptible than the white. There is no description given of the disease. It is doubtful that the purple leaf spot is the same as Ajrekar's blight. Evidence will be presented later to show that the pathogene with which we are concerned in the present case is not necessarily a wound parasite. It has already been stated that red varieties are as susceptible as the white.

The purple leaf spot was first reported simultaneously by extension agents from Barros and Vega Baja, Porto Rico, during the month of December, 1924. Material for study from those places was received at the Experiment Station at Rio Piedras during the same month. Early in January, 1925, the disease appeared in an experimental plot at the Experiment Station and furnished excellent material for further study.

*Economic Importance*

The disease caused considerable damage to the 1924–25 crop in Barros and Vega Baja and greatly reduced the yield. In one instance the writer observed the almost complete destruction, during rainy weather, of a large planting where the disease appeared before the bulbs had begun to form.

The disease may be expected to be one of increasing importance in the island since the onion promises to become a more important crop in Porto Rico in the near future than it has been up to this time.

### *Symptomatology*

The disease attacks both the leaves and flower stalks. The first symptoms are numerous tiny, white, circular or irregular spots, less than one millimeter in diameter. These gradually increase in size until in advanced stages the diseased areas cover several square centimeters of surface. The daily progress of the lesion into the healthy tissues is marked by a new zone of freshly discolored tissues. As the spots increase in size, they become oval-shaped or irregular and the incipient white color eventually changes to violet.[3] Later stages of development show the central portion of the spots changing to a Pompeian red, dark Indian or Perila purple, immediately surrounded by a pale yellow orange to salmon band beyond which is a pale green zone. Dull violet-black zones within the lesions are also to be observed. In many instances these colors blend into different shades, spreading over the whole surface of the lesion. The dark purple color is the most distinctive symptom of the disease. A distinct yellowing usually extends from both ends of the spots, often reaching the tips and bases of the leaves. In very old spots the purple or violet color gradually fades out, and a pale ochraceous-buff develops, the center retaining some shades of purple. Similar lesions are produced on the flower stalks. The lesions on the latter cause girdling in most cases, and as a rule the stalks are destroyed before the seeds are mature.

Brown conidia of the pathogene soon appear in abundance, borne at the end of dark colored conidiophores on the surface of the lesions, giving a brown tinge to the spots.

The spots on the false shallot are similar to those on the onion but they are smaller and the color is somewhat paler. The purple color is not so pronounced as on onion leaves.

### *Etiology*

#### *Classification and identity of the causal organism*

*Taxonomic relations.* The original basis for distinguishing between the genera *Alternaria* and *Macrosporium* is the manner of spore production, *i.e.*, whether they occur singly or in chains. Those forms producing spores in chains have been generally placed under the genus *Alternaria*, while those occurring singly, irrespective of morphology, have been considered to

[3] Throughout this paper references to colors are based on Ridgway's "Color Standards and Nomenclature."

belong to *Macrosporium*. That there is on this basis no sharp line of demarkation is now a well-established fact. There are forms which agree in every other respect with the morphologic characters of *Alternaria* but do not ordinarily produce spores in chains. The question then arises as to the genus under which this particular species is to be included.

Elliott (4) published an account of his investigations on these two genera. He states that "all spores of the obclavate, cuneate, or ovate form produce chains of spores under favorable conditions." He also states that "there is no doubt that all of the species with these types of spores belong to the genus *Alternaria*, that most of the species named under *Macrosporium* belong to this genus, and can be recognized as such by the descriptions given of their spores." He adds further that "where species of *Alternaria* have been described the spores have been more or less elongated and pointed at one end." This fungus appears to exhibit all the morphologic features of an *Alternaria* as that genus is defined by Elliott. In referring the species to this genus the writer has, therefore, accepted Elliott's opinion that "all obelavate, cuneate, ovate, pointed or beaked spores belong to *Alternaria*."

As to the specific identity of this pathogene, it can only be pointed out that it apparently differs morphologically from other so-called Macrosporium and Alternaria species heretofore described as occurring on onions. A comparison of the description of the fungus, given below, with that of *Macrosporium parasiticum* Thüm. (13) shows them to be quite distinct. The latter has oblong-ovate to depressed, rotund spores always rounded at both ends as contrasted with the oblong-clavate and beaked spores of this species. Moreover, the spores of *M. parasiticum* are minutely warted (8, p. 3, pl. III) while those of this species are smooth. It is clear that they are not even generically identical, *M. parasiticum* being a true *Macrosporium* in the sense of Elliott.

While *M. porri* Ellis is undoubtedly an *Alternaria*, it has much smaller spores than those of this species. Moreover, its spores have simple beaks, judging from the description by Saccardo (13) and the illustrations by Thaxter (15, figs. 40–43). A large number of the spores of the species under consideration possess compound beaks. It may be identical with the *Alternaria* which Ajrekar (1, 2) believes to be the cause of an onion leaf blight in India but, as he gives no description of his fungus or the symptoms caused by it, one cannot assume them to be identical. On the basis of such evidence as is available it seems wisest to call the purple leaf spot pathogene a new species which is here designated *Alternaria allii*.

**Alternaria allii** sp. nov. Mycelium in the lesions hyaline or brown; in culture white to smoky, reddish or fuliginous, blackish-green or olive; 4–10 μ

thick in the lesions, sometimes attaining a thickness of 18 μ in culture; composed of smooth, septate, short, simple or branching, subfasciculate hyphae; in culture media, cells often much constricted at the septa, frequently separating smoothly at these constrictions, containing distinct refractive droplets; chlamydospore-like bodies and gemmae present in cultures. Conidiophores arising through the stomata or breaks in the epidermis as terminal branches of hyphae; solitary or fasciculate; erect or decumbent; septate; fuliginous to dark brown or even brown olivaceous; blunt at the tips; 20–180 × 4–18 μ, basal cells or segments usually wider than the terminal portion of the sporophore. Conidia borne singly at the apex of the conidiophore; elongate-clavate, apically attenuate to form a long beak which is often branched; multiseptate, muriform; 105–320 × 12–24 μ; at first hyaline, brown or dark olivaceous when mature; the broad base of the spore 6–12 septate with one or two longitudinal septa in the middle segments of some; slightly constricted at septa; beak continuous or several-septate; germinating very readily in water; spore production in culture media exceedingly scanty or none.

Hab. On leaves of *Allium cepa* L. in Porto Rico.

*Pathogenicity of Alternaria allii.* Microscopic examination of the lesions shows the constant association of a fungus of the Alternaria type. The affected tissues are invaded by a ramifying, septate, brown mycelium from which arises over the necrotic area of the spot an abundance of typical large muriform spores (Plate III, 11–22).

*Cultural characters.* Pure cultures were obtained from typical spots on the leaves of onions by plating out spores in agar or by tissue plantings.

All carbohydrate media were sterilized in the Arnold steam sterilizer for one hour on three consecutive days. All other media were autoclaved for 30 minutes at 20 lbs. pressure. The pathogene was grown six times in each of the culture media, using pedigree cultures, and detailed observations were made daily on changes which occurred. All cultures were grown at room temperature, which ranged around 78° F. during the period when this work was carried out.

On oatmeal agar the fungus makes a very profuse growth. The mycelium is dark with a reddish to violaceous tinge. The substratum is cadmium-orange. Masses of aerial mycelium later become amethyst-violet to hyacinth-violet. A dusky violet blue tint is often observed in the mycelial growth.

On Cook's II agar[4] the mycelium is profuse, smoky white to dark dull gray. The substratum is black except in the region lying just below the

[4] Preparation: 20 gms. agar agar; 10 gms. peptone; 20 gms. glucose; 0.5 gm. dipotassium phosphate and 0.5 gm. magnesium sulphate; water 1,000 cc.

outer growing ring of the mycelium which is light-brown, changing later to hazel brown.

On Czapeck's agar[5] the organism makes excellent and extremely rapid growth. The aerial mycelium is profuse, mostly mineral red with a smoky white outer growing zone. The substratum is of a more or less Mars violet color.

On nutrient agar[6] growth is moderately fair; aerial mycelium brown or gray. The substratum does not change in color.

On non-nutrient agar growth is very poor, scanty and loose; aerial mycelium white.

On saccharose (3 per cent) agar growth is fairly good; aerial mycelium loose, whitish, and the substratum sulphine yellow.

On dextrose (3 per cent) agar the substratum is olive green; aerial mycelium greenish; growth fair.

On lactose (3 per cent) agar growth is fair; substratum olive green; aerial mycelium loose, dark.

On dextrine (3 per cent) agar the substratum is aniline yellow; aerial mycelium whitish; growth fairly good.

On maltose (3 per cent) agar the substratum is yellowish-oil; aerial mycelium greenish, loose.

On cornmeal agar the growth is profuse; aerial mycelium ochraceous-buff to yellow ochre with violaceous spots scattered throughout the surface; substratum Mars violet. A few spores appeared at the end of 4 days.

On Thaxter's hard potato-dextrose (2 per cent) agar aerial mycelium is profuse, varying in color from ochraceous buff to olivaceous black and an intermediate violet color. A few spores were found at the end of 3 days.

On hard potato saccharose (2 per cent) agar the outer growth of mycelium is yellow ochre; older inner mycelium aconite violet; substratum Morocco red to blackish. A few spores were found at the end of 3 days.

On hard potato-lactose (2 per cent) agar the mycelium produces abundant whitish aerial tufts, although the rest of the aerial mycelium is dark gray; substratum vinaceous; tawny under young growth and black under the old growth.

On hard potato-maltose (2 per cent) agar the growth is similar to that on potato-saccharose agar but spores are not produced; aerial mycelium profuse, violaceous to purple.

A comparative study of the rate of growth on sugar and sugar-starch media in petri-dish cultures showed at the end of 7 days for lactose and dextrine (3 per cent) agars, a thallus diameter of 6.5 cm.; for saccharose,

[5] Prepared by adding 10 gms. agar agar to 500 cc. Czapeck's solution.

[6] Prepared by adding 10 gms. agar agar to 500 cc. bouillon consisting of 2.5 gms. Liebig's beef extract, 2.5 gms. sodium chloride and 500 cc. distilled water.

dextrose, maltose (3 per cent) and cornmeal agars a diameter of 7.5 cm.; for potato-saccharose, potato-dextrine and potato-maltose (3 per cent) agars a diameter of around 8.5 cm. while on potato lactose (3 per cent) agar the thallus reached a diameter of 9 cm.

On scales of yellow onion bulbs cut in pieces and steamed in petri dishes in the autoclave, growth is excellent, producing a dense white mass of mycelium at the end of two days. This later changes to a rather olivaceous or reddish color. The color of the scales changes to a reddish or olive color. This color reaction approaches that observed on the tissues of the suscept under natural conditions in the field and confirms the opinion that such color effects are entirely due to this fungus.

On pieces of onion leaves steamed in test tubes in the autoclave, the fungus produces abundant and dense masses of white mycelium.

In bouillon (−15 Fuller's scale) growth is fairly good and abundant smoky gray mycelium is produced.

Czapeck's solution gives the best growth of all liquid media tried. A dense mycelial felt covers the surface of the liquid. The superficial mycelium is violaceous, the submerged is dark brown to black.

On Cohn's solution growth is fair, mycelium whitish.

On onion decoction the mycelium is dense and abundant as in Czapeck's solution. A purplish tinge is noticeable in the superficial mycelium while the liquid becomes filled with dark brown mycelium.

Summary of cultural characteristics on these media:

1. The organism makes profuse growth in all carbohydrate media.
2. Growth is best on oatmeal agar, cornmeal agar, Czapeck's agar, Cook's II agar, and potato-sugar media.
3. Growth is relatively poor on sugar media not containing starch or proteid.
4. Growth on onion tissues and decoctions is fairly good.
5. The fungus causes a change in color of the sterilized onion scales, which is similar to that produced on the suscept under natural conditions.
6. Best growth in liquid media was observed in Czapeck's solution.

In cultures, especially in carbohydrate media, the pathogene itself exhibits marked color variations. Ravn (12, pp. 101–327), in studies of the genus *Helminthosporium*, shows that the plasma and cell-walls may be blackish-green, grayish-brown, or entirely black as in the genus *Alternaria*. Teodoro (14, p. 242) working with *M. parasiticum* Thüm., states that the mycelium is at first hyaline but later assumes a smoky white to light, yellowish-brown color and later becomes deep brown and greenish. Similar colorations have been observed in the mycelium of *A. allii* in culture.

The smooth mycelium branches easily and profusely in water and culture media, anastomosing into a compact, intricate, heavy white mass. Neighboring hyphae sometimes anastomose.

Drops of slightly-colored liquid separate from the body of the fungus and collect on the surface. The nature of these drops was not investigated. Chlamydospore-like bodies and gemmae have been found in old cultures, a character observed in *Helminthosporium* by Mitra (7).

Abnormalities have been observed in the mycelium under various treatments. Ten-day old cultures on potato-dextrose agar were flamed to produce a slight injury to the superficial mycelium. The plates were then set aside and aerial mycelium was allowed to grow. Abnormal swellings in the injured hyphae were induced, this occurring very frequently in the end cells, in some cases suggesting the early stages of spore formation. In other cases small, more or less irregular or elongated structures were seen to arise on the sides of cells.

*Effect of environmental factors on growth in culture.* Experiments with different concentrations of acid and alkali demonstrate that *Alternaria allii* makes its maximum growth in media of slightly acid reaction. It was also found that the fungus will tolerate larger concentrations of alkali than of acid.

To ascertain whether light has any effect on growth, two sets of plate cultures on Czapeck's agar were arranged as follows: 10 plates were wrapped in heavy black paper of the kind used for photographic purposes (each plate separately) and were kept in the dark at room temperature. Another set of the same number was kept in the light. In the plates kept in the dark there was slightly less aerial mycelial growth but color relations were much the same as in case of the plates kept in the light. The difference is insignificant or almost negligible.

To determine the effect of quantity of medium on growth, two sets of six plates each were poured with Czapeck's, Cooks' II, and oatmeal agars, one set containing 12 cc. and the other 24 cc. of medium per plate respectively. Transfers were made from pure cultures to the center of each plate. The plates were kept in the light at room temperature. The results were recorded daily for a period of seven days.

It was found that growth is uniformly greater in the larger quantity of medium. Aerial mycelium was much denser, producing a heavy hyphal mat over the surface of the plates containing the double quantity of medium. No differences in color of mycelium or substratum were observed.

To determine the effect of inhibitory influences on production of spores and character of thallus growth, transfers of the fungus from pure culture were made to Cook's II agar plates making the transplant at the center of the plate. Three transplants of bacteria (species not determined) and one

of *Aspergillus sp.* were planted on the border of the agar close to the edge of the plate. It was observed that the thallus growth of the *Alternaria* was inhibited and checked in the direction of the other plantings. The medium under the regions where the fungus was approaching these plantings became cadmium to lemon or chrome in color, contrasting with the dark gull gray of that under the rest of the thallus.

To determine the effect of moisture on growth, rice tubes were prepared using a small quantity of water in one case, in another using double the quantity. In the same way onion leaves were steamed in test tubes. The fungus was grown for six days in these tubes and the results were observed. In tubes with the least water the growth was rapid but limited. In tubes with the larger quantity of moisture growth was dense, profuse, heavy with abundance of white aerial mycelium.

A great variety of culture media has been used in an effort to obtain spores of the fungus under purely artificial conditions. The methods employed by Rands (10) and Kunkel (5) have been tested. Teodoro (14, pp. 242, 267–268) was successful in obtaining conidia in potato-dextrose agar with *Macrosporium parasiticum* Thüm. All attempts to induce conidial production have been unsuccessful. In these studies a few spores of *A. allii* have been obtained in 3- or 4-days old cultures in oatmeal, potato-dextrose, and hard potato-saccharose agars but spore production soon ceased and was never abundant. The only explanation which suggests itself for this phenomenon is that the mycelium of this species exhibits an excessive tendency toward hyphal growth at the expense of spore production. This is especially true of media with high moisture content. There is undoubtedly a delicately balanced relation between spore production and air humidity over the growing colony.

The spores produced in cultures are considerably smaller than those which occur under natural conditions. They very rarely exhibit the characteristic long beak or isthmus, this remaining more or less rudimentary. The conidia germinate in cultures as soon as they are produced.

Summary of effect of environmental factors:

1. The fungus tolerates larger concentrations of alkali than of acid. Best growth is obtained in slightly acid media.
2. The amount of light does not have much influence on thallus growth.
3. There is much more growth with the greater amount of medium.
4. Colonies of other fungi and bacteria show inhibitory influences on the growth of *Alternaria allii*.
5. Large quantities of moisture in the medium increase the abundance of white aerial mycelium.

*Infection experiments.* The inoculum used throughout the infection experiments was mycelium from single spore cultures used in the preceding studies and spores taken from natural lesions.

Six healthy plants growing in clay pots under field conditions were sprayed with a spore-suspension of the fungus. The pots were then covered with bell jars lined with moist filter paper. Two days later the first symptoms of the disease appeared as small, whitish necrotic areas. A second set of six plants was sprayed with a suspension of bits of mycelium from 6-day old cultures and covered with bell jars. The symptoms of the disease appeared on the second and third days. A third set of plants was inoculated by transferring spores of the fungus to needle-pricks made on the surface of the leaves. A rapid dying of the adjacent tissues was observed on the second day. A fourth set of plants was inoculated through needle-pricks on the leaves with bits of mycelium from 6-day old cultures of the fungus. Dying of the surrounding tissues was observed on the second day. Check plants wounded but not inoculated showed no further evidences of injury.

Similar inoculation experiments were conducted in the greenhouse. The fungus was repeatedly reisolated from lesions produced by artificial inoculation.

These experiments together with the constant association of the fungus with the lesions establish beyond doubt the ability of *Alternaria allii* to cause the disease of onions under consideration.

Inoculation with spore suspensions gave slightly less prompt infection than that with growing mycelium from cultures. Infection was found to be most prompt when the inoculum was applied to wounds in the leaves.

*Life History*

In Porto Rico, onion seed is sown in seed beds and the seedlings transplanted into the field. Seed beds are generally started during the latter part of August, through September, and even during early October. Transplanting is done during October and early in November. At this time there is heavy rainfall in some of the onion districts and moderate showers in others. The average period of growth after setting of transplants in the field is three and one-half months under normal conditions (9). The harvesting season begins in December and lasts through January.

This pathogene has a very simple life history, living for the relatively short period of pathogenesis on the green leaves and seed stalks of the onion, and continuing its existence as a saprogene on the debris of its suscept after the death of these organs.

The primary infections usually occur just prior to bulb formation, about the time the plants show the greatest vigor. The source of inoculum is most probably the debris of diseased onions left on the field from the previous crop, as onions are commonly grown year after year in the same field.

Conidia produced during the previous season do not appear to serve as inoculum for the primary cycles. This is indicated by germination studies in which it was found that spores germinate promptly after they are produced, whenever moisture is present. This is further confirmed by the desiccation experiments from which it appears that the conidia do not withstand much drying and so soon lose their viability.

In the experiments to determine the effect of desiccation on the vitality of the spores, four drops of a spore suspension in distilled water were transferred to each of 60 glass slides. The water was then evaporated by means of an electric fan. The slides were divided into two lots, A and B, of 30 slides each. Lot A was placed in a moist chamber with the humidity maintained as near saturation as possible. Lot B was placed in a dry chamber, *i.e.*, at the prevailing humidity of the laboratory air. The room temperature was around 78° F. during the period of this experiment. Each day after these experiments were initiated, one slide from each lot was removed and a drop of sterile distilled water placed over each of the four spore smears on the slide. These were then placed in moist chambers to germinate. One hundred per cent germination occurred in slides from lot A during the first 7 days and in lot B during the first 5 days. Germination rapidly dropped to 50 per cent in both lots by the 12th day. On the 15th day lot A gave a germination of 33 per cent while lot B gave but 10 per cent germination. No germination was obtained from lot B after the 18th day, while lot A continued to give a rapidly declining germination until the 27th day, after which the spores would no longer grow. It is thus evident that if under the ordinary dry conditions of the laboratory spores are killed within 18 days, in the hot sun of the open fields they probably succumb in a very short time. Even a saturated atmosphere will not keep them alive for a month. Periods of high humidity under field conditions, while frequent, are usually of relatively short duration due to the trade winds which blow daily.

Since but one crop of onions is grown during the year, spores produced on the lesions on living plants will all have perished in one way or another before the next crop is growing. So only those conidia which are produced from mycelium in the old debris can serve as primary inoculum.

These conidia are transferred to the infection courts by spattering rains and possibly by thrips. Conidia may also be blown by the wind but this is probably of rare occurrence in the case of primary inoculations. The leaves are the only infection courts.

Conidia deposited on the leaves soon germinate if a film of water is present. The germ tubes enter through the stomata, and through wounds made by thrips (*Thrips tobaci* Lind.) or by other agents. A stomatal vesicle has not been observed to be produced by the germ tubes. Soon after entrance

the germ tubes branch in all directions and grow intercellularly. Incubation is completed and invasion accomplished in a few hours.

The first evidences of infection appear in from 1 to 4 days as small bleached or whitish spots. The mycelium branches and spreads in all directions through the tissues, killing all the adjacent cells. Growth and development of the pathogene is very rapid during the early stages of infection, especially when the plant is in an actively growing condition. The progress of the pathogene is accompanied by yellowing and bleaching of the invaded tissues, which is brought about by the destruction of the chloroplastids. The necrosis of the chloroplastids is followed by plasmolysis of the cytoplasts and by oxidation discoloration of the cell walls and cell contents.

Conidiophores bearing conidia begin to develop over the necrotic area of the lesion about the fifth day after the first symptoms appear. The leaves bearing these primary lesions soon shrivel and dry. They are shortly broken away by the wind or in cultivation and fall to the ground, where the fungus continues a saprophytic existence for a time, producing conidia for further infections.

Conidia produced on the primary lesions serve as the inoculum for secondary infections. They are produced in great numbers and are readily detached from the conidiophores. They are carried to the infection courts by the wind, spattering rain, and probably by thrips. Since the distance between plants in the field is never greater than 10 by 10 inches, spattering rain is probably the chief agent in conveying the inoculum. However, strong winds bring healthy leaves in contact with diseased ones and thus they become inoculated, especially when wet with rain or dew. In these ways the fungus is rapidly disseminated throughout the field.

Spore germination has been carefully studied. The conidia for these studies were taken from natural lesions in the field, since, as already pointed out, conidia have not been readily obtained in culture. Spore suspensions were made by scraping conidia from the lesions and transferring them to 5 cc. of the liquid medium in which they were to be germinated. Germination was studied in tap water, rain water, distilled water, beef bouillon, Czapeck's solution, Cohn's solution, onion leaf decoction and onion scale decoction. All studies were made in inverted drops of these media in Van Tieghem cells held at room temperature. The spore suspensions were started about 9 A. M. and were examined first at 5 minute intervals, later every 2 minutes.

All spores began to germinate at the end of 21–45 minutes. Just before germ tubes begin to appear the contents of the cells in some cases contract. The constriction between the cells of the conidium becomes more pronounced due to increased turgor of the cells. This is especially true in the case of

the cells of the beak (Plate IV, 26 and 28). The first definite indications of germination are slight protrusions in the cell walls which rapidly develop into definite germ tubes. A septum is soon formed in each tube a short distance from the germinating cell. At the end of three hours practically every cell of the basal part of the spore has usually germinated, as well as some of those of the beak (Plate IV, 26 and 28). The germ tubes by this time are all abundantly septate (Plate IV, 28). The width of the tubes at this time is from 3.4 μ to 4.3 μ, and branching has already begun.

A comparative study of spore germination as it progressed in the different media above listed gave some results probably worthy of record. The length of the germ tubes at the end of 2 hours was approximately the same in all media except distilled water, in which they were appreciably longer. At the end of 4½ hours they were all about the same length except in beef bouillon, where they were distinctly shorter. In tap water, rain water, distilled water and Cohn's solution there was little tendency to branching of the germ tubes even at the end of 6 hours, although their length was as great as in the other media. This probably was due to a lack of available nutrients in the media, since in those cases growth is largely dependent on the food stored in the spore.

In bouillon, Czapeck's solution, and in decoctions of onion leaves and scales, the germ tubes were profusely branched and abundantly septate at the end of 6 hours, indicating the absorption and use of the nutrients in these media.

In onion scale decoction and onion leaf decoction, germination was slightly slower than in non-nutrient solutions and showed a tendency to marked septation. Trials to determine the effect of different strengths of the onion leaf or scale decoctions on germination were conducted, using varying amounts of tissue in a given amount of water. The results show a slight inhibitory or deleterious influence of the higher concentrations.

On solid media it was observed that in general the germ tubes are wider, shorter, and more constricted at the septa, exhibiting profuse branching.

Invasion of the uninjured leaf by the germ tubes appears always to be by way of the stomata. Repeated observations fail to show any case of direct penetration through the epidermal cells. The invading hyphae spread through the intercellular spaces of the leaf. Actual penetration of the cells themselves has never been observed. Hyphal tips emerge through stomata or in some cases rupture the epidermis to give rise to the conidiophores.

Various types of conidiophores have been observed in this species. Among the most common is a short septate, finger-like conidiophore borne at the end of a fertile hypha singly or in groups (Plate III, 3, 7 and 8 and Plate V, 33–39, 46–47). It is hyaline to light or dark brown and blunt at the tip. The end cells of the vegetative hypha become enlarged or swollen

and constitute the basal cells from which the conidiophore arises. The number of these swollen tips varies from one to four or five at each point of emergence. This type of conidiophore has extremely heavy walls, and like the mycelium it becomes filled with refractive drops or is abundantly vacuolate.

The second type of conidiophore is characterized by thinner walls, is longer and slightly narrower (Plate III, 1–2, 4–6). It occurs in abundance in very old lesions, and the basal cell is not so swollen as in the preceding type. It is closely septate and dark brown in color. Old conidiophores of this type show ovoid to globular or irregular small cells at their ends. This probably indicates the formation of conidia successively on a conidiophore, each corresponding to a detached conidium (Plate III, 9). Clusters of conidiophores usually arise from one, two or three swollen cells in the fertile hypha (Plate V, 60).

Conidia develop from the uppermost cell of the sporophore. At first they are small, hyaline, one-celled and long-ovate (Plate V, 50). The next step is marked by a slight growth in width and pronounced growth in length; septation begins. The walls now become slightly colored. At this stage conidia are two- to three-celled, and the apex becomes slightly attenuated (Plate V, 51–53). The base becomes enlarged and the apex greatly elongated. The base now becomes several-celled and light brown, the protoplasm taking on a more granular appearance while the beak remains hyaline. The spores vary in form, some being club-shaped while others are flask-shaped (Plate V, 54–58). The number of cells in the base increases rapidly, the spore takes on a decided brown coloration throughout the large basal portion and the apical cell becomes fully elongated into a hyaline beak or isthmus which is often branched (Plate V, 59), though there are always some spores with simple beaks only (Plate III, 14, 16–21).

A large majority of the older conidia possess compound or branching beaks. This peculiar branching of the beaks, which has been observed in *Alternaria radicina* Meier, Drechsler and Eddy (6, p. 163, fig. 2) and in *A. solani* (E. and M.) J. and G. by Rands (11, fig. 4) seems to occur once or twice in individual spores, resulting in two or four branches, as the case may be, arising from a single central beak (Plate III, 13, 15, and 22). Even six branches may be encountered in some cases. The occurrence of branched or compound beaks in these conidia presents a sharp contrast between this species and others of the genus *Alternaria* where conidia are borne in chains. Catenulation of spores has been sought in vain, both in nature and in culture. It evidently does not normally occur, if at all, in this pathogene.

The middle portion of the spore is usually wider and the cells are more numerous in this region. The walls of the spores are thick, as are the walls of the conidiophores. Two types of conidia differing as to size were observed in some of the lesions, some decidedly smaller than the usual large

type (Plate III, 16–21). Isolations from these spores were made. Isolations were also made from another specimen showing conidia of the usual large size (Plate III, 11–15). Field inoculations were properly carried out using both strains. Both produced similar lesions. Later examinations revealed the fact that each strain was capable of producing spores of either size. It may therefore be concluded that they belong to the same species and are only variations. What may be the conditions bringing about these variations in spore size has not been investigated.

Successive secondary cycles continue the destruction of new foliage as fast as it develops, preventing the filling or development of the bulbs. The blighted leaves shrivel and fall to the ground, where the fungus continues its activities under favorable moisture conditions until the next crop of onions is planted and presents growing foliage for the initiation of another period of pathogenic activity.

### *Epiphytology*

The ideal field conditions for epiphytotic development of the disease appear to be warm weather with occasional rains. Just such weather conditions prevailed during January, 1925, in one experimental plot at the Experiment Station, and the disease was most destructive. There was a very vigorous, luxuriant growth at the time the disease first appeared. In seven days the pathogene had infected all the plants. Light afternoon rains favored the development of the pathogene, which soon destroyed the greater part of the foliage. Heavy losses were reported from another section of the northern part of the island where conditions were similar to those prevailing in the Experiment Station grounds. The disease made a violent attack during December, 1924, in the central part of the island, where the humidity of the air was high. Continued rains occurred and there was little sunshine.

The relation of light to spore germination and its bearing on time of invasion of the leaves was studied. All tests were made with spores in hanging drops of distilled water in Van Tieghem cells. Germination at night occurred promptly in 27–40 minutes and was vigorous and normal. In tests made at 7 A. M. in the shade, the spores began to germinate in 21–35 minutes; septation and branching followed rapidly. In short, early morning germination paralleled in every respect that which took place at night. To determine the effect of direct sunlight on spore germination, parallel sets of spore suspensions were set up at 8 o'clock in the morning, one in the shade, the other exposed to the direct rays of the sun. Those in the shade began to germinate in 21–32 minutes; those in the sunlight in from 27 to 43 minutes. Three hours after the spores were placed in suspension, measurements of germ tubes were made in each set. Those in the shade averaged 166μ, while in the direct sunlight the maximum was 9μ. It is evident therefore that, while diffused light has little or no effect on promptness or

vigor of germination, the direct rays of the sun markedly retard growth of the germ tubes or even prevent their formation. It seems clear, then, that spore germination and invasion of the leaf, or in other words incubational activity, occurs only during the night and early hours of the morning or during cloudy weather. Showers during the day followed by bright sunny weather is evidently not favorable to infection.

## CONTROL

No field experiments on the control of this disease have been undertaken. Preliminary studies on the toxic effects of copper sulfate were carried out. Spore suspensions were made in solutions of copper sulfate in concentrations of 1:100, 1:1,000, 1:10,000, 1:100,000 and 1:1,000,000 in Van Tieghem cells. Hourly observations were made during the first day and the final results recorded at the end of two days. The spores germinated normally in dilutions of 1:10,000 and higher, but germination was almost completely inhibited at 1:1,000.

It would appear from these tests that *Alternaria allii* is relatively resistant to copper, the results indicating a susceptibility to copper poisoning about equal to that of *Alternaria solani* as determined by Doran (3, p. 535). While no tests of the fungicidal efficiency of copper in Bordeaux mixture against *A. allii* have been made, it is doubtful that it will prove effective, especially in view of the difficulties of making it spread and adhere to the glaucous surface of onion leaves. There would seem to be some promise of success from the use of copper dusts of high copper content, especially if the copper be in colloidal form.

Sanitary practices involving the removal and destruction of diseased crop debris promise little of practical value because of the rapidity with which the diseased leaves break and crumble as soon as they shrivel and dry. Rotation of the crop where that is feasible should be of some value in holding the disease in check.

## SUMMARY

1. A new Alternaria disease of onions occurring in Porto Rico is described.

2. The market onion (*Allium cepa* L.) and the false shallot (a viviperous variety of *Allium cepa* L.) are affected.

3. The disease is characterized on the onion leaf by dark purple, oval-shaped or irregular spots.

4. The symptoms on the false shallot are essentially like those on the onion except for a paler color of the spots.

5. The pathogene, a typical *Alternaria*, is described as a new species *Alternaria allii*. It is characterized by the branched beaks of its conidia.

6. The cultural characters of the causal fungus on a variety of media were studied. Growth on carbohydrate media is excellent in general, being

better in starch-containing media than in media containing sugars alone. Color changes are induced in all solid media including sterilized onion leaves and bulb scales; also in onion decoctions. An acid reaction of the medium is more favorable to growth than alkalinity. Light does not affect rate of mycelial growth in artificial media. Thalli of certain other fungi and colonies of certain bacteria inhibit growth of *A. allii*.

7. All attempts to induce abundant spore production in pure culture failed. A few spores were observed in some cultures.

8. Infection experiments fully establish the pathogenicity of *A. allii* on the leaves of growing onions. Wounds are not necessary for invasion. Entrance of the germ tubes is normally through the stomata.

9. The conidia germinate in water in 21–45 minutes at ordinary room temperature as soon as mature. They are short-lived, being very susceptible to drying and direct sunlight. Germination and invasion apparently occurs at night or in cloudy weather and only in the presence of meteoric water.

10. The pathogene apparently lives over from one onion crop to the next as mycelium in onion leaf debris on the soil, from which a crop of conidia for the primary infection is produced.

11. The conidia are relatively resistant to copper sulfate, being able to germinate in a 1:10,000 solution. A 1:1,000 solution prevents germination.

DEPARTMENT OF PLANT PATHOLOGY,
CORNELL UNIVERSITY,
ITHACA, N. Y.

LITERATURE CITED

1. AJREKAR, S. L. Report of the work done under the plant pathoiogist to the Government of Bombay, Poona, for the year 1920–21. Bombay Dept. Agr. Ann. Rept. **1920–21**: 121–122. 1922.
2. AJREKAR, S. L. Annual report of the work done under the plant pathologist to Government of Bombay, Poona, for the year 1921–22. Bombay Dept. Agr. Ann. Rept. **1921–22**: 102–104. 1923.
3. DORAN, W. L. Toxicity studies with some copper fungicides. Phytopath. **13**: 532–542. 1923.
4. ELLIOTT, J. A. Taxonomic characters of the genera *Alternaria* and *Macrosporium*. Amer. Jour. Bot. **4**: 439–476. 1917.
5. KUNKEL, L. O. A method of obtaining abundant sporulation in cultures of *Macrosporium solani* E. and M. Brooklyn Bot. Gard. Mem. **1**: 306–312. 1918.
6. MEIER, F. C., C. DRECHSLER and E. D. EDDY. Black rot of carrots caused by *Alternaria radicina* n. sp. Phytopath. **12**: 157–166. 1922.
7. MITRA, M. *Helminthosporium* spp. on cereals and sugar cane in India. Part I (Diseases of *Zea mays* and *Sorghum vulgare* caused by a species of *Helminthosporium*). Memoirs Dept. Agr. India, Agr. Res. Inst., Pusa. **11**: 219–242. 1923.
8. MIYABE, KINGO. On the life history of *Macrosporium parasiticum* Thüm. Ann. Bot. **3**: 1–25. 1889.

9. NOLLA, J. A. B. El cultivo de la cebolla en Puerto Rico. Porto Rico Dept. Agr., Bur. Ext. Circ. 9: 1–22. 1925.
10. RANDS, R. D. The production of spores by *Alternaria solani* in pure culture. Phytopath. 7: 316–317. 1917.
11. RANDS, R. D. *Alternaria* on *Datura* and potato. Phytopath. 7: 327–338. 1917.
12. RAVN, F. K. Nogle Helminthosporium—arter og de af dam Fremkaldte Sygdomme hos Byg og Havre. Bot. Tid. 23: 101–327. 1900.
13. SACCARDO, P. A. *Macrosporium parasiticum* Thüm. Sylloge Fungorum 4: 537. 1886.
14. TEODORO, NICANOR G. A study of a Macrosporium disease of onions (*Allium cepa* L.). Philipppine Agr. Rev. 16: 227–280. 1923. (Also as publication of Dept. Agr. and Nat. Resources, Bureau of Agriculture, P. I. 1923.)
15. THAXTER, ROLAND. The larger onion *Macrosporium* (*Macrosporium porri* Ell.). Conn. Agr. Exp. Sta. Ann. Rept. 1889: 161–162. 1890.

## EXPLANATION OF PLATES

### PLATE III

Conidia and conidiophores of *Alternaria allii*

1–2, 4–6, 10. Typical large conidiophores.

3. Young conidiophores borne at branched apex of a fertile hypha.

7. Conidiophores arising from a single fertile hypha underneath the epidermis.

8. Fasciculate cluster of conidiophores.

9. Old conidiophore showing cells formed at apex as a result of successive spore formation.

11–12. Conidia attached to conidiophores.

13–15, 22. Typical large conidia with branched beaks.

16–21. Small conidia with simple beaks.

### PLATE IV

Germination of conidia of *Alternaria allii*

23, 24, and 29. Conidia germinating after a few days desiccation; showing production of secondary conidia at end of short, fertile germ tubes.

25 and 30. Young conidia germinating.

26. Cells of beak germinating. Note constriction at septa.

27 and 31. Extent of germination in distilled water after 21 minutes and 40 minutes, respectively.

28. Germinated conidium at the end of 3½ hours in distilled water. Note constriction at septa of beak.

### PLATE V

Development of conidiophores and conidia of *Alternaria allii*

32 and 60. Conidiophores arising from enlarged cells at the apices of hyphae.

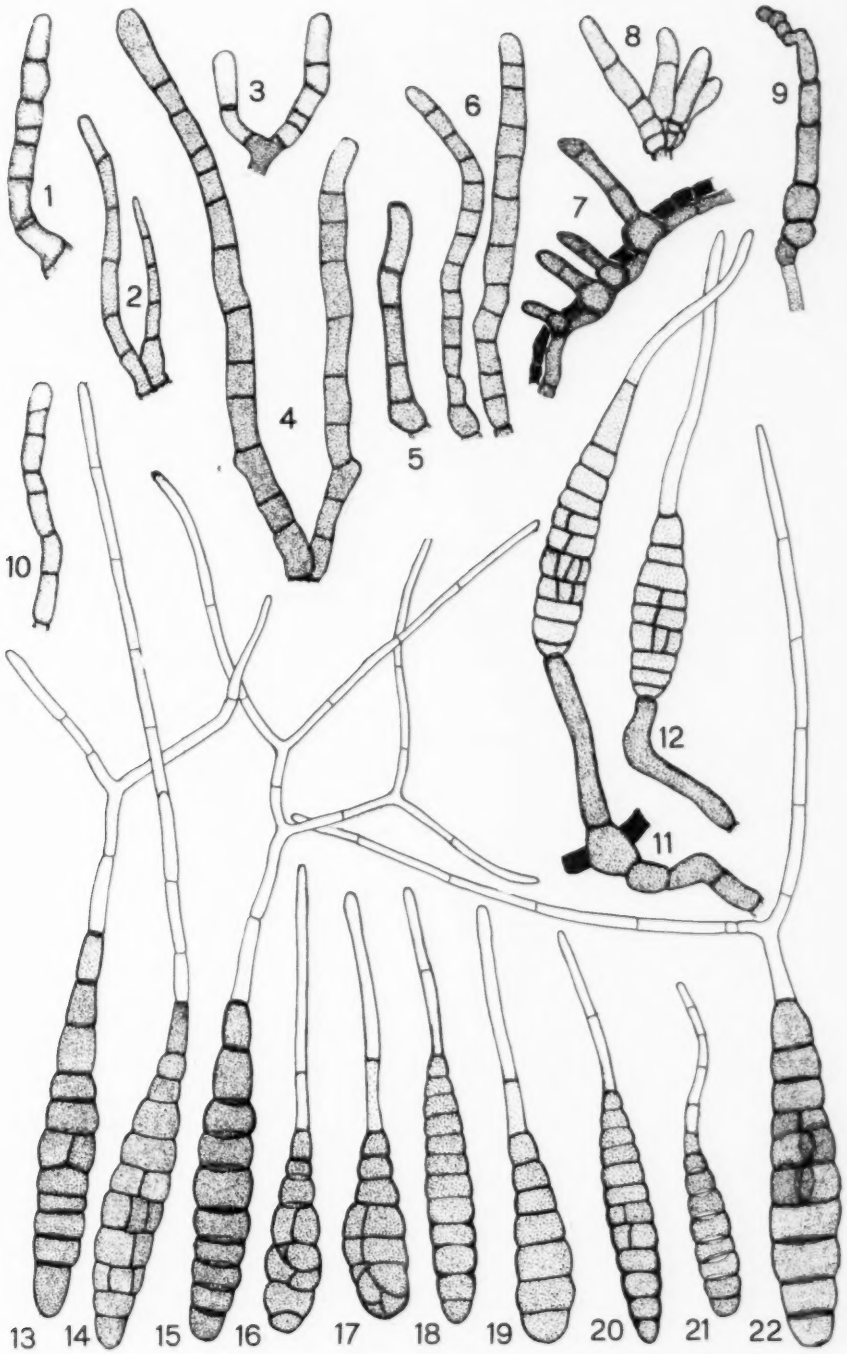
33–49. Various stages in development of conidiophores.

50–54. Early stages in conidial development.

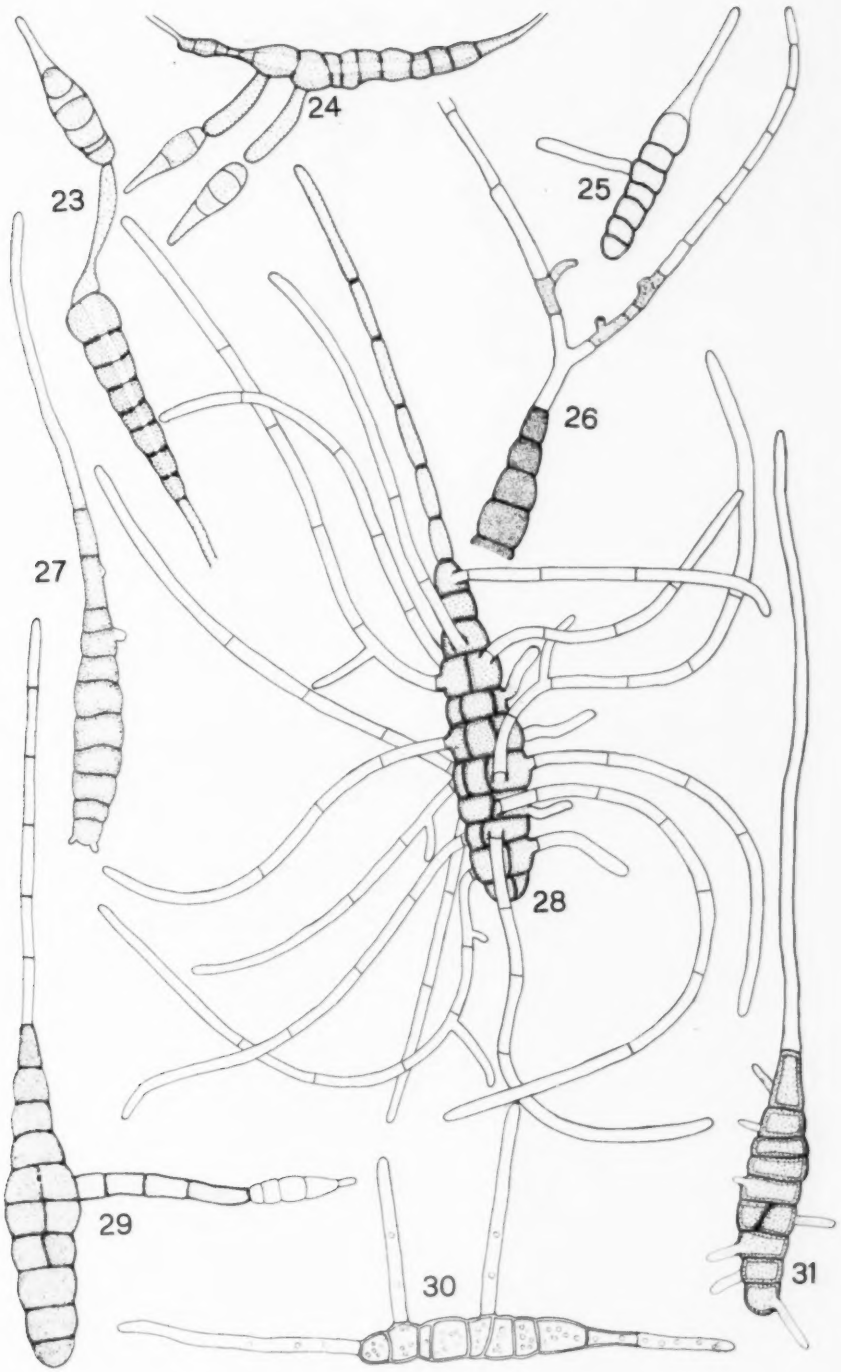
55–58. Primary septation of conidia and early stages in development of beak.

59. Final stage of conidial development, lower part of spore becoming septate and brown, beak becoming septate and branched.

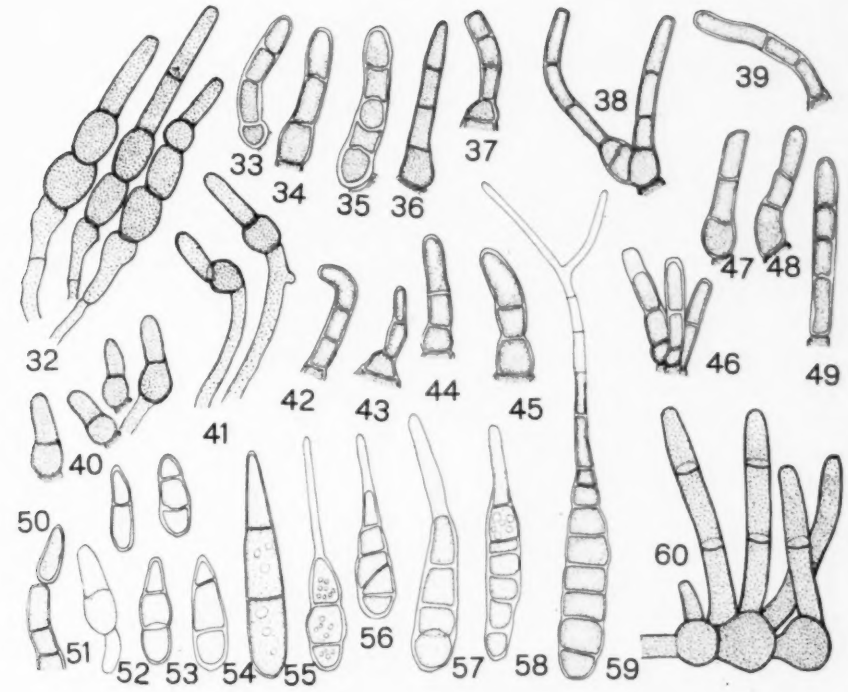
61. Lesions of the purple leaf spot of onions.


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22

UNIV OF MICH


24
23
25
26
27
28
29
30
31

UNIV. OF MICH.


32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

61

UNIV. OF MICH.

# ADDITIONAL HOSTS OF APHANOMYCES EUTEICHES, THE PEA ROOTROT FUNGUS

MAURICE B. LINFORD

Observations in Wisconsin pea fields and experimental plantings in the greenhouse have shown that the pea rootrot fungus, *Aphanomyces euteiches* Drechsler, has a wide range of potential host plants, and is not limited in its parasitism to *Pisum sativum*.

In the summer of 1925, narrow-leaved vetch was found with characteristically decayed roots containing oospores typical of this fungus in several fields of peas in two localities in Wisconsin. During 1926, seedlings of alfalfa, sweet clover, and an undetermined species of grass were found similarly diseased, and roots of barley and oats were found to contain oospores, apparently of this fungus. Several varieties of sweet peas were seen at McMillan, Michigan, much weakened by the attack of this pea rootrot fungus. Roots of red clover, alsike clover, white clover, and several weeds were examined in thoroughly infested fields but were not found to be affected.

Of fifteen species of leguminous plants grown from seed in naturally infested soil (Colby silt loam) in the greenhouse in the winter of 1925–26, the following proved susceptible: *Medicago sativa, Melilotus alba, Lathyrus odoratus, L. latifolius, Lathyrus* sp. (native of northern Utah), *Vicia sativa, V. pannonica, V. monantha, V. gigantea, V. fulgens, V. ervilia, V. dasycarpa,* and *V. angustifolia*. Two species, *V. villosa* and *V. colcarata*, were not infected. An undetermined species of monocotyledonous bulb plant found by chance in the soil used in this trial also developed this rootrot disease. No pure culture inoculations have been made, but the morphology of the oospores of *A. euteiches* is sufficiently characteristic to make possible the rather positive identification of the parasite.

All of these hosts are relatively resistant in comparison with peas, as shown by lower percentages of plants infected, by less extensive invasion of infected roots, and by the absence of visible infection excepting when grown in wet soils particularly favorable for the disease. In the case of alfalfa and sweet clover, only young roots, before secondary thickening has disturbed the primary cortex, seem susceptible to attack. Very young alfalfa seedlings in diseased pea fields have been seen badly weakened and even killed by this disease, but such plants have constituted at most only a small fraction of the stand. In greenhouse trials, certain species of *Vicia* and

*Lathyrus* proved nearly as susceptible as peas, some of these seedlings being killed shortly after emergence. In general, species with coarse roots seem more susceptible than those with very fine roots.

The present list of hosts is such as to suggest that *Aphanomyces euteiches*, given particularly favorable conditions, may live as a weak parasite in the roots of a wide range of plants, not restricted to the Leguminosae. This supports the hypothesis that it may occur naturally in some soils prior to the first growth of peas, but as yet the rootrot fungus has not been found under virgin conditions in the roots of native plants. Rootrot has been observed several times in Wisconsin in recently cleared ground where peas were planted for the first time. The remarkably long persistence of the parasite in soils once thoroughly infested may now be readily understood. This wide range of hosts makes it apparent that rotation of crops with whatever interval between crops of peas cannot be confidently relied upon to eradicate the rootrot fungus from infested soil, even though experience has shown a long rotation to be a valuable aid in reducing the severity of infestation.

UNIVERSITY OF WISCONSIN,
MADISON, WISCONSIN.